GINSENG RADIX

Ginseng

DEFINITION

Ginseng consists of the whole or cut dried root of *Panax ginseng* C.A. Meyer (Araliaceae). It contains not less than 0.4 per cent of combined ginsenosides Rg_1 ($C_{42}H_{72}O_{14}.2H_2O$; M_r 837) and Rb_1 ($C_{54}H_{92}O_{23}.3H_2O$; M_r 1163), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

Triterpene saponins of the dammarane type, as derivatives of either protopanaxadiol or protopanaxatriol. Examples of protopanaxadiol saponins are: ginsenosides Ra_1, Ra_2 and Ra_3; ginsenosides Rb_1, Rb_2 and Rb_3; ginsenosides Rc and Rd; and malonyl-ginsenosides Rb_1, Rb_2, Rc and Rd. Examples of protopanaxatriol saponins are: ginsenosides Re and Rf; 20-gluco-ginsenoside Rf; ginsenosides Rg_1, Rg_2 and Rh_1. Ginsenoside Ro is a derivative of oleanolic acid.

The ginsenosides considered the more important are ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1 and Rg_2, with Rb_1, Rb_2, Re and Rg_1 being the most abundant. The total ginsenoside content of a 6-year-old main root varies between 0.7 and 3%. The lateral roots can contain two to three times more saponins than the main root while the slender roots can contain up to 10 times more [2-10].

Other constituents include peptidoglycans called panaxans, acetylenic compounds such as panaxynol, pyrazine derivatives such as 3-*sec*-butyl-2-methoxy-5-pyrazine, oligo- and polysaccharides, phenolic compounds such as vanillic acid and salicylates, and traces of essential oil containing sesquiterpenes such as eremophilene [8,9].

CLINICAL PARTICULARS

Therapeutic indications

Decreased mental and physical capacities such as weakness, exhaustion, tiredness and loss of concentration, as well as during convalescence [11-16].

Posology and method of administration

Dosage

Adult daily dose: 0.5 g up to a maximum of 2 g of dried root; equivalent preparations [2,11,13,14].

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Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen after one month, medical advice should be sought.

Contra-indications
None known.

Special warnings and precautions for use
Do not exceed the recommended dose.
Diabetics should consult a physician prior to taking ginseng root [17].

Interaction with other medicaments and other forms of interaction
Ginseng intake may slightly reduce blood glucose levels [17].

A case of possible interaction of ginseng with warfarin anticoagulant therapy has been reported, but the mechanism remains unknown; studies are needed to verify this potential interaction and the underlying mechanism [18]. In rats, concomitantly administered ginseng had no significant effect on the pharmacokinetics or pharmacodynamics of warfarin [19].

Pregnancy and lactation
In animals, no effect on fetal development has been observed. No human data are available [20].

In accordance with general medical practice, ginseng should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
On the basis of ginseng's long-term usage and the relative infrequency of significant demonstrable side effects, it has been concluded that the use of ginseng is not associated with serious adverse effects if taken at the recommended dose level [20,21].

Oestrogenic-like side effects have been reported in both pre- and post-menopausal women following the use of ginseng. Seven cases of mastalgia in post-menopausal women after ingestion of ginseng products of unspecified botanical origin have been reported [22-24]. However, in clinical studies with more than 100 patients to whom a standardized ginseng extract had been given, no oestrogenic-like side effects have been observed and only normal hormone blood levels have been found in the specific tests carried out in these studies [25,26].

An *in vitro* study showed that the concentration of ginseng extract or ginsenosides needed for competitive inhibition of the binding of promegestone to the cytosolic progesterone receptor of human myometrium is far higher than the ginsenoside concentrations which can be reached after oral administration [27].

Overdose
Critical analysis of a report on a so-called Ginseng Abuse Syndrome [28] has shown that there were no controls or analysis to determine the type of ginseng ingested or the constituents of the preparation taken, and that some of the amounts ingested were clearly excessive (as much as 15 g, whereas the recommended daily dose is 0.5 to 2 g daily) [20,29,30]. The only conclusion that can validly be drawn from the above report is that excessive and uncontrolled intake of ginseng products should be avoided [20].

One case of ginseng-associated cerebral arteritis has been reported in a patient consuming 200 ml of a preparation made from 12.5 g (dry weight) of ginseng and 200 ml of rice wine [31].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

A review of the major findings of pharmacological tests and human studies carried out with a number of plant drugs including ginseng supported the view that ginseng enhances physical performance and learning capacities and has immunomodulatory properties [32].

In vitro experiments

Cell proliferation
In experiments with human male fetal lung fibroblasts (MRC-5), addition of ginseng extract (dialyzed or heat treated to inactivate protein-precipitating activity) to the culture medium resulted in the following:

- up to 100% increase in cell density at concentrations equivalent to 0.05-2.0 mg whole root/ml medium and decreased cell density at more than 5.0 mg/ml.
- 0.75 mg extract/ml had an effect on cell density equivalent to that of 5 µg hydrocortisone/ml.
- faster growth rate, smaller and more mitotic cells and longer survival time without media change after addition of 0.75 mg/ml or after addition of 5 µg hydrocortisone/ml [33].

Activation of biosynthesis
Addition of a fraction from a ginseng extract to a homogenate of rat testes stimulated DNA and protein synthesis. The effect was reduced by addition of the enzyme inhibitor cycloheximide [34].

A standardized ginseng extract stimulated D-glucose transport in Ehrlich ascites tumour cells and D-glucose uptake in rabbit cerebral cortical tissue [35,36].

Hormone receptor binding
Binding of the radioactive-labelled sex hormone promegestone to the cytosolic progesterone receptor of human myometrium in the presence or absence of ginseng extract or pure ginsenosides was investigated. The results demonstrated that the concentration of ginseng extract or ginsenosides needed for competitive inhibition of the binding of promegestone to the receptor were far higher than the concentrations which can be reached after oral administration [31].

Immunomodulation
Peripheral blood mononuclear cells from healthy volunteers (n = 20) or from patients with chronic fatigue syndrome (n = 20) or AIDS (n = 20) were tested, in the presence or absence of varying concentrations of ginseng extract, for natural killer cell activity against K562 cells and for antibody-dependent cellular cytotoxicity against human herpes virus 6-infected H9 cells. Ginseng extract in concentrations of 1, 10 and 100 µg/ml significantly ($p<0.05$ to $p<0.001$) enhanced cellular immune function of peripheral blood mononuclear cells from all groups [37].

The mitogenic activity and anticomplement activity of a standardized ginseng extract and several fractions from it were tested in mice spleen cell cultures. It was found that the extract possessed anticomplement and mitogenic activities, the strongest anticomplement activity being observed in a crude polysaccharide fraction. The polysaccharide with the major anticomplement activity consisted of arabinose, galactose and glucose with small amounts of galacturonic acid, glucuronic acid and rhamnose; its molecular weight was estimated to be 3.68×10^5 kD [38].

An acidic polysaccharide fraction from ginseng containing galactose, arabinose and uronic acids inhibited *Helicobacter pylori*-induced haemagglutination with a minimum inhibitory concentration of 250 µg/ml. Digestion of the fraction with pectinase resulted in a lower molecular weight oligosaccharide fraction which was non-inhibitory at 4 mg/ml [39].

Ex vivo experiments

Antioxidant properties
Two groups of 5 rats were given ginseng extract as 10 mg/ml in drinking water (corresponding to 1.6 g/kg/day) for one week then one group was exposed to hyperbaric 100% oxygen (HBO) for 6 hours; two control groups (no ginseng extract) drank an equal amount of water and one control group was exposed to HBO. Isolated perfused hearts from the rats were subjected to mild ischaemia and then reperfused. The results indicated that the ginseng extract prevented myocardial ischaemia/reperfusion damage and the impairment of endothelial functionality induced by reactive oxygen species arising from HBO exposure, through an antioxidant intervention [40].

In experiments with perfused rabbit lungs, a ginseng extract inhibited vasoconstriction induced by the thromboxane analogue U46619 or by acetylcholine after exposure to free radicals generated by electrolysis. This effect appeared to be due to the release of nitric oxide from the pulmonary endothelium. The extract had activities superior to pure ginsenosides. Moreover, pure ginsenosides at higher concentrations appeared to have a negative effect rather than a protective one [41,42].

Immunomodulation
After oral administration of a standardized ginseng extract to mice at 10 mg/day for 4 consecutive days the effect on immune response was investigated. The extract enhanced antibody plaque forming cell response and circulating antibody titre against sheep erythrocytes [43].

The above finding was confirmed by experiments involving oral administration to mice of a ginseng extract with defined ginsenoside content. Oral administration at 10, 50 or 250 mg/kg body weight daily for 5-6 days resulted in enhanced immune responses in a battery of 6 *ex vivo* tests which included primary and secondary immune response against sheep red cells, natural killing activity, mitogen-induced proliferation, interferon production and T-cell mediated cytotoxicity [44].

In vivo experiments

Humoral effects
Intraperitoneal administration of ginseng saponin fractions to rats stimulated adrenocorticotrophic hormone (ACTH) secretion from the hypothalamus/hypophysis, leading to increased corticosterone synthesis and excretion [45,46].

Effects on performance
Intraperitoneal administration to rats of a 1% ginseng extract (20 mg extract/kg body weight twice with a 24-hour interval) led to significant differences in energy metabolism after exercise (swimming for 60 minutes) as measured by determination of plasma concentrations of relevant metabolites. Glucose was higher ($p<0.01$) and free fatty acids were lower ($p<0.01$) after 30 minutes, whereas pyruvic acid ($p<0.05$) and lactic acid ($p<0.01$) were lower after 60 minutes compared to saline treated controls. It was concluded that ginseng extract shifted the skeletal muscle energy metabolism towards oxidation of fatty acids, thereby conserving carbohydrate stores [47].

The effects of a standardized dry ginseng extract

administered intraperitoneally to rabbits were investigated using electrocorticograms. The results indicated that the extract stimulated metabolic activity of cerebral tissue. Incubation of sliced rabbit brain tissue with the same extract (23 and 46 µg/ml) led to highly significant metabolic changes ($p<0.0005$ compared to control values): glucose uptake increased, while lactate and pyruvate production and the lactate/pyruvate ratio decreased. The effects appeared to be dose-dependent [36].

Oral administration of a standardized ginseng extract to rats for 3 months at 3, 10 or 100 mg/kg body weight per day produced dose-dependent, significant increases in performance in the treadmill test and diminished hepatic lipid peroxidation compared to controls ($p<0.05$) [48,49].

After oral administration of a standardized ginseng extract, rats were subjected to learning and memory retention tests and ^{14}C-phenylalanine transport across the blood-brain barrier was also determined. After intraperitoneal injection of rats with the extract the brain stem and brain cortex were analysed for concentrations of monoamines and 3',5'-cyclic adenosine monophosphate (AMP), and for the activity of phosphodiesterase and adenylate cyclase. The following results were obtained:

- Improved learning and memory retention (5 of 7 tests) after oral administration of the extract at 20 mg/kg body weight for 3 days, but unchanged or even decreased learning and memory retention (4 of 7 tests) after 100 mg/kg orally for 3 days.
- Increased ^{14}C-phenylalanine transport across the blood-brain barrier after 30 mg extract/kg orally for 5 days.
- Unchanged phosphodiesterase activity in brain stem and cortex after 50 mg extract/kg intraperitoneally for 5 days.
- Decreased adenylate cyclase activity (with or without NaF-activation) after 30 mg and 200 mg extract/kg intraperitoneally for 5 days, except after 30 mg extract without NaF-activation when adenylate cyclase activity was increased.
- Decreased 3',5'-cyclic AMP concentration in brain stem and cortex after 200 mg extract/kg intraperitoneally for 5 days.
- Increased dopamine and noradrenaline concentrations in brain stem, whereas the serotonin concentration was decreased in brain stem and increased in brain cortex, after 50 mg extract/kg intraperitoneally for 5 days.

This study thus revealed the influence of ginseng extract on complex neurological processes such as learning and memory as well as on several aspects of brain metabolism [50].

Oral versus intraperitoneal administration of a standardized ginseng extract was compared in a study involving tests for exhaustion by swimming in mice and cold stress resistance in rats. Physiological and biochemical tests (body weight increase, food and water consumption, urine analysis for sodium, potassium and chloride, liver total cholesterol, total lipids and triglycerides, adrenal total cholesterol, blood and serum glucose, triglyceride and cholesterol values) were also carried out. Significant effects ($p<0.05$) seen in this study were:

- A prolongation of time to exhaustion in mice after a single intraperitoneal administration of ginseng extract. Oral administration of a daily dose corresponding to 37.5 mg/kg body weight for 15 days also produced an effect.
- Body temperature under cold stress in rats was higher after intraperitoneal administration of ginseng extract or 5 mg ACTH per kg body weight compared to controls receiving saline solution. Hydrocortisone (10 mg/kg) did not have this cold-protective activity. In adrenalectomized rats on the other hand, ginseng extract did not have cold-protective activity whereas hydrocortisone did.
- In a study in weanling rats the fresh weight of thymus was reduced after intraperitoneal administration of ginseng extract for 4 days in doses corresponding to 1.5-30 mg of ginsenosides/kg. The magnitude of effect was dose-dependent.
- Liver protein, total cholesterol and total lipids were reduced in male and female rats after 15 days of intraperitoneal administration of extract in doses corresponding to 3 or 30 mg ginsenosides/kg body weight/day. Such changes were not seen after 15 days oral administration of extract doses corresponding to 1.87 or 37.5 mg ginsenosides/kg body weight/day except for a reduction of liver protein in female rats receiving the higher oral dose. Adrenal total cholesterol was markedly reduced only in orally treated animals. Furthermore, body weight increase was monitored weekly in a prolongation of the same experiment comparing 5 weeks oral administration with 4 weeks intraperitoneal administration. Growth retardation was seen in male rats both after oral and intraperitoneal treatment but was stronger and dose-dependent after intraperitoneal treatment. In female rats weak and non-dose-dependent growth retardation was only seen after intraperitoneal treatment. It was concluded that ginseng acts indirectly on the level of central humoral regulation via the adrenocortical system [51].

Groups of 7-week-old male Swiss mice (15 per group) received, as their only liquid for up to 96 days, either distilled water or an infusion from ginseng corresponding to 33 mg of dry root powder/ml. The average consumption per mouse corresponded to daily

ingestion of 274 mg of root powder. No significant differences between treatments were seen in weight gain or in the cold swimming test after 35, 46 or 96 days [52].

In another study rats received by stomach tube, 48 hours and 1 hour before a warm water swimming test, crude saponins from 6 different types of ginseng at 50 mg/kg body weight. Swimming time until drowning, plasma levels of lactic acid, glucose, insulin and glucagon, and liver glycogen, were measured in both resting and drowned animals. The only significant differences ($p<0.05$) were an increased plasma level of glucose in drowned rats that had received ginseng. Plasma glucose levels were higher in all resting rats that had received saponins but the differences were within normal biological variability [53].

In a study of antioxidant activity, liver lipid peroxide levels following ethanol intoxication were determined in mice which had received oral doses of various ginseng root constituents (maltol, salicylic acid, vanillic acid, coumaric acid and ginsenosides Rb_1, Rb_2, Rc, Re, Rg_1) at 0.001-1 mg per 30 g body weight for 3 days before the test. Maltol, salicylic acid and vanillic acid decreased liver lipid peroxide levels strongly while saponins had a weaker effect and coumaric acid was inactive [54].

Immunomodulation
Daily subcutaneous administration to thymectomized rats of an extract corresponding to 25 mg ginseng/kg body weight for 10 days gave significant protection ($p<0.04$ to $p<0.004$) against intratracheal challenge with alginate-embedded *Pseudomonas aeruginosa* [55].

Anti-ulcer effects
Oral administration to rats of 50, 200 or 500 mg/kg body weight of a 70%-methanol extract from ginseng inhibited in a dose-dependent manner gastric ulceration induced by pyloric ligation, serotonin or endotoxin, but did not inhibit stress-induced ulceration. Serotonin- or endotoxin-induced effects on gastric mucosal tissue blood flow were reduced. Most effects observed after doses of 200 mg/kg or 500 mg/kg were statistically significant ($p<0.05$ or $p<0.01$) [56].

Hepatoprotective effects
Intraperitoneal administration of a single dose of a ginseng extract to rats at 50 mg/kg body weight did not protect the animals against CCl_4-induced hepatotoxicity [57].

Subcutaneous injection of rats with dexamethasone (0.5 mg and 1.0 mg/day) for 7 days, alone or in combination with a ginseng extract (corresponding to 100 mg of root/day) for 5 days, demonstrated that the extract reduced dexamethasone-elevated alanine amino transferase (ALAT) and aspartate amino transferase (ASAT) levels to normal [58].

Pharmacological studies in humans

Performance
In a double-blind crossover study, 12 student nurses working night shifts (3-4 consecutive nights followed by 3 days of rest) were given 1.2 g of ginseng or placebo for the first three consecutive nights of night work and tested on the morning after the third night. Crossover medication was given after an interval of at least 2 weeks. A third series of tests was carried out during normal daytime working, after no medication and following a good night's sleep (GNS). The subjects assessed their mood, physical well-being and degree of lethargy by means of linear self-rating scales; two psychophysiological performance tests and haematological tests were also carried out. The detrimental effects of night shifts on mood and performance were clearly seen. A constant trend in favour of ginseng compared to placebo was noted. Ginseng ratings were favourable for mood criteria, but unfavourable for physical well-being criteria. Ginseng restored blood glucose levels raised by night shift stress to the GNS level. It was concluded that ginseng had a small but consistent anti-fatigue effect [11].

Various tests of psychomotor performance were carried out in a group of 16 healthy male volunteers given a standardized ginseng extract (2 × 100 mg daily for 12 weeks) and in a similar group given placebo under double-blind conditions. A favourable effect of ginseng relative to baseline performance was observed in attention (cancellation test), processing (mental arithmetic, logical deduction), integrated sensory-motor function (choice reaction time) and auditory reaction time. However, end performance of the ginseng group was statistically superior ($p<0.05$) to the placebo group only in mental arithmetic. No difference between ginseng and placebo was found in tests of pure motor function (tapping test), recognition (digit symbol substitution) and visual reaction time [12].

In a double-blind, placebo-controlled, crossover study, 43 top triathletes received either 200 mg of a standardized ginseng extract or placebo daily for periods of 10 weeks. Significant differences ($p<0.05$) in various endurance parameters were seen only after the second treatment phase. It was concluded that ginseng improved endurance (resistance to end of season stress) but did not improve optimum performance [13].

Twenty top class male athletes received 200 mg of a standardized ginseng extract daily for 9 weeks. In the bicycle ergometer exercise test lasting 8 minutes, post-treatment values were higher for maximal oxygen

absorption and lower for blood lactate level and heart rate during exercise compared to pretreatment values. The differences were significant ($p<0.001$) [14].

In a double-blind study athletes were given 200 mg of ginseng extract standardized to 7% ginsenosides (n = 10) or 200 mg of ginseng extract standardized to 4% ginsenosides + 400 mg of vitamin E (n = 10) or placebo (n = 10) daily for 9 weeks. Using the same bicycle ergometer test, significant differences were observed in favour of either of the two ginseng preparations compared to placebo with respect to heart rate ($p<0.05$), blood lactate ($p<0.01$) and maximal oxygen absorption ($p<0.01$) after exercise. Differences between the two ginseng preparations were not significant. Levels of testosterone and luteinising hormone in plasma and of free cortisol in urine were unchanged after all treatments [14,15].

A double-blind, placebo-controlled study involving 28 trained male athletes examined the persistence of effects of a 9-week treatment (200 mg of ginseng extract with 4% ginsenosides, or placebo) beyond the treatment period. Compared to placebo the ginseng extract produced significant improvements in maximal oxygen uptake during exercise ($p<0.01$), heart rate at maximal exercise ($p<0.001$), forced expiratory volume ($p<0.01$), forced vital lung capacity ($p<0.05$) and visual reaction time ($p<0.01$). These positive effects lasted for at least 3 weeks after treatment. It was concluded that the effects of ginseng are based on clinically relevant metabolic changes, which persist for a certain period after treatment [16].

In a double-blind, placebo-controlled study involving 50 ambulant patients suffering from asthenia, depressive syndrome or neurovegetative disorders, the effects of 8 weeks of daily treatment with 200 mg of a standardized ginseng extract on performance were evaluated by two psychometric tests and from the results of a comprehensive psychological questionnaire (Sandoz Clinical Assessment Geriatric). Significant improvement ($p<0.05$ and $p<0.01$) was seen in most of the parameters [59].

In a randomized, double-blind study, healthy male volunteers received 200 mg (n = 11) or 400 mg (n = 10) of a ginseng extract, or placebo (n = 10), daily for 8 weeks. The extract had no effect on oxygen consumption, respiratory exchange ratio, minute ventilation, blood lactic acid concentration, heart rate or perceived exertion [60].

In another randomized, double-blind study, healthy female volunteers received 200 mg of a ginseng extract (n = 10) or placebo (n = 9) daily for 8 weeks. Ginseng had no effect on maximal work performance or on resting, exercise and recovery oxygen uptake, respiratory exchange ratio, minute ventilation, heart rate or blood lactic acid levels [61].

In a double-blind, placebo-controlled, crossover study involving 8 healthy volunteers (mean age 25 years) who regularly practised physical activities, 30 days of oral treatment with 400 mg of a standardized ginseng extract per day did not improve performance in supramaximal exercise (125% of the maximum aerobic power on a bicycle ergometer), nor did it influence blood lactate or blood testosterone [62].

In a study of the blood oxygenation status of 8 male and 2 female middle-aged subjects (average age 50 years), a significant increase ($p<0.05$) in resting arterial pO_2 was observed after 4 weeks of daily oral treatment with 200 mg of a standardized ginseng extract; the resting arterial pO_2 increased by 4.5 mm Hg. In synergy with oxygen treatment the increase was 10.1 mm Hg. Venous pO_2 decreased (4.3 mm Hg) [63].

The effects of 400 mg/day of a ginseng extract for 8-9 weeks on a variety of cognitive functions were compared with placebo treatment in a randomized, double-blind study involving 112 healthy volunteers older than 40 years (55 verum, 57 placebo). The ginseng group showed a tendency to faster simple reactions and significantly better abstract thinking than the controls. However, there was no significant difference between groups in concentration, memory or subjective experience [64].

The effects of a standardized ginseng extract on psychological mood states and perceptual response to submaximal and maximal exercise stress were evaluated in a study involving 19 young adult females who received either 200 mg of a standardized ginseng root extract (n = 10) or placebo (n = 9) daily. The results did not support claims that ginseng can enhance psychological function characteristics at rest and during exercise stress [65].

The effects of a standardized ginseng extract (300 mg/day) on healthy, untrained male students and on healthy male students who received regular bicycle ergometer training were compared to placebo in an 8-week randomized, double-blind study (n = 41). Administration of the ginseng extract produced training-like effects on VO_2 max. and on anaerobic power and leg muscle strength, but no synergistic effect on these fitness variables occurred when administration of ginseng extract was combined with exercise training [66].

Immunomodulation

The effects of ginseng on immune parameters were studied in a randomized, double-blind study in which groups of healthy volunteers of both sexes, aged between 18 and 50 years, were treated orally with 2 × 100 mg of a standardized ginseng extract (n = 20) or 2 × 100 mg of a dry aqueous extract from ginseng (n = 20) or placebo (n = 20) daily for 8 weeks.

- Standardized ginseng extract increased the chemotaxis of circulating polymorphonuclear leucocytes ($p<0.05$ at week 4 and $p<0.001$ at week 8), increased the phagocytosis index and phagocytosis fraction ($p<0.001$ at weeks 4 and 8), increased total lymphocytes (T3) ($p<0.05$ at week 4 and $p<0.001$ at week 8), increased the T-helper subset (T4) ($p<0.05$ at week 4 and $p<0.001$ at week 8), increased the helper/suppressor (T4/T8) ratio ($p<0.05$ at weeks 4 and 8), enhanced induction of blastogenesis in circulating lymphocytes ($p<0.05$ at weeks 4 and 8 after induction by cocanavalin A and pokeweed mitogen; $p<0.001$ at weeks 4 and 8 after induction by lipopolysaccharide) and enhanced natural killer cell activity ($p<0.05$ at week 4 and $p<0.001$ at week 8).

- Aqueous ginseng extract increased the chemotaxis of circulating polymorphonuclear leucocytes ($p<0.05$ at weeks 4 and 8), increased the phagocytosis index and phagocytosis fraction ($p<0.05$ at week 8), increased total lymphocytes (T3) ($p<0.05$ at week 4 and $p<0.001$ at week 8), increased the T-helper subset (T4) ($p<0.05$ at week 8), enhanced induction of blastogenesis in circulating lymphocytes ($p<0.05$ at week 8 after induction by cocanavalin A and pokeweed mitogen) and enhanced natural killer cell activity ($p<0.05$ at week 8).

- With placebo, only enhancement of natural killer cell activity was significant ($p<0.05$) after 8 weeks.

It was concluded that ginseng extracts act as immunostimulants in man and that the standardized extract was more active than the aqueous extract [67,68].

Healthy volunteers enrolled in a multicentre, randomized, double-blind, placebo-controlled study to investigate potential effects of ginseng on resistance to influenza and the common cold were treated with 200 mg of a standardized ginseng extract ($n = 114$) or placebo ($n = 113$) daily for 12 weeks. All participants received an anti-influenza polyvalent vaccine at week 4. Results from examinations at weeks 4, 8 and 12 showed highly significant differences ($p<0.0001$) between the ginseng extract and placebo with regard to the frequency of influenza or colds between weeks 4 and 12 (15 cases in the verum group versus 42 cases in the placebo group). Antibody titres at week 8 were also much higher after verum treatment (272 units versus 171 units after placebo) and natural killer cell activity in the verum group was almost twice as high as in the placebo group [69].

In a controlled single-blind study to investigate the effects of a standardized ginseng extract (200 mg/day) in 40 patients suffering from chronic bronchitis the extract significantly ($p<0.001$) improved alveolar macrophage activity compared to baseline [70].

In a pilot study involving 15 patients with severe chronic respiratory diseases, a standardized ginseng extract was administered orally at 200 mg/day for 3 months and respiratory parameters such as vital capacity, expiratory volume and flow, ventilation volume and walking distance were evaluated. The results led to the conclusion that ginseng extract improved pulmonary function and oxygenation capacity, which seemed to be the reason for improved walking capacity [71].

A study in two groups of 10 healthy young Thai males evaluated the effects of 300 mg of a standardized ginseng extract daily for 8 weeks in comparison with placebo on peripheral blood leukocytes and lymphocyte subsets. No significant differences were observed [72].

In a first attempt at a systematic review of some of these studies it was suggested that further investigations are needed to conclusively establish the efficacy of ginseng [73].

Pharmacokinetic properties

Pharmacokinetics in animals

A study involving intravenous, intraperitoneal and oral administration of purified or semipurified ginsenosides Rg_1, Rb_2, Rd and Re in rabbits gave the following results:

- The pharmacokinetic behaviour of some ginsenosides is best described by a one-compartment open model.
- Ginsenoside Rb_2 had a longer elimination half-life (445 minutes) and lower metabolic and renal clearance than ginsenosides Rg_1 and Re, obviously due to a higher rate of plasma protein binding.
- Absorption into the systemic circulation after intraperitoneal administration was slow for all the ginsenosides studied.
- No ginsenosides were found in plasma or urine samples after oral administration; analysis of faecal samples for ginsenoside Rg_1 also gave a negative result [74].

The pharmacokinetics of ginsenoside Rg_1 were studied in rats, comparing oral and intravenous administration. Rapid absorption of 1.9-20.0% of orally administered Rg_1 (t_{max} 30 minutes) and rapid excretion of intravenous Rg_1 (almost 60% of the dose in bile within 4 hours and 24% in urine within 12 hours) were detected by TLC [75].

The pharmacokinetics of ginsenoside Rb_1 were studied

in rats after oral or intravenous administration. Orally administered Rb_1 was very poorly absorbed (approx. 0.1%) from rat intestine. Excretion of Rb_1 after intravenous administration was biphasic with a half-life of 11.6 minutes for the α-phase and 14.6 hours for the β-phase. Excretion was mainly in the urine (44% within 120 hours) and poorly in bile (0.8% within 24 hours) [76].

Decomposition products of ginsenosides Rb_1 and Rg_1 in the rat gastro-intestinal tract have been investigated. The pattern of decomposition products in rat stomach was similar to hydrolysis products under mild acidic conditions except for one compound. Decomposition products in the large intestine were due to the activitiy of enteric bacteria and an enteric enzyme [77].

The metabolites of ginsenosides are poorly investigated with regard to pharmacokinetics as there are more than 10 ginsenosides, each giving rise to at least 5 different metabolites. A study in mini-pigs following intravenous administration of Rb_1 and Rg_1 confirmed findings in rats and rabbits. Rb_1 was excreted biexponentially (two-compartment open model) with a half-life of 16 hours in the β-phase (20 minutes in the α-phase). Rg_1 pharmacokinetics are best explained by a one-compartment model, the elimination half-life being 27 minutes [78].

A study in mice involving intravenous or oral administration of tritiated [^{3}H]ginsenoside Rg_1 indicated rapid absorption after oral administration (appr. 30% after 1 hour). Relatively high concentrations were found in blood, liver, bile, subcutis, conjunctiva and epithelia of oral and nasal cavities and oesophagus, whereas the concentration in muscle and endocrine organs was low. Addition of total ginseng extract or of a purified ginsenoside fraction to oral administration of tritiated Rg_1 did not change the distribution pattern of Rg_1. Excretion of intact Rg_1 in faeces and urine was low, whereas excretion of metabolites was high [79].

After oral administration of ginsenoside Rb_2 to rats, six decomposition products were found in the large intestine by TLC. On the basis of ^{13}C-NMR, five decomposition products resulted from stepwise cleavage of sugar moieties [80].

The decomposition of ginsenoside Rb_2 in rat stomach was compared to its decomposition in 0.1N HCl. There was little decomposition in the stomach with only small amounts of 24- and 25-hydroxy- and -hydroperoxy-derivatives being found. Decomposition in 0.1 N HCl solution yielded two main derivatives due to cleavage of a sugar moiety [81].

A further study investigated the decomposition of ginsenosides Rb_1 and Rb_2 in rat stomach and large intestine, in 0.1 N HCl and in crude hesperidinase solution. The results confirmed a quantitatively small decomposition to hydroxy- and hydroperoxy-derivatives in rat stomach, whereas decomposition in 0.1 N HCl yielded derivatives arising from cleavage of sugar moieties. It was also found that the decomposition of Rb_1 and Rb_2 by cleavage of sugar moieties in rat large intestine was partly due to bacteria and partly due to enteric enzymes such as β-glucosidase. Different decomposition products were found in rat large intestine compared to those found after treatment with hesperidinase [82].

Pharmacokinetics in humans

Dose-dependent urinary excretion of 20(*S*)-protopanaxatriol glycosides (1.5% of the dose) was observed in 4 healthy volunteers after oral ingestion of ginseng powder and ginseng extract preparations corresponding to 6.2-27.6 mg of ginsenosides per day [83].

Using selective ion-monitoring GC-MS, ginsenoside aglycones were quantified in urine from athletes who claimed to have consumed ginseng preparations for at least 10 days before urine collection. Aglycone concentrations of 2-35 ng/ml were found in 60 out of 65 urine samples [84].

Cleavage of sugar moieties from ginsenosides Rb_1, Rb_2, Rc and Rd by intestinal bacteria isolated from human faecal samples has been demonstrated. *Prevotella oris* was identified as the major bacterial species with this ability [85].

Preclinical safety data

A 1984 review [20] summarized the results of several toxicity studies of a standardized ginseng root extract in animals:

Single dose toxicity

- The oral LD_{50} was determined as > 5 g/kg body weight in the rat, > 2 g/kg in the guinea pig and > 1 g/kg in mice.
- The intraperitoneal LD_{50} was > 1 g/kg in rats and mice.
- After oral administration of the ginseng extract to mini-pigs at 0.25, 0.5 or 2.0 g/kg body weight no noticeable changes were observed in cardiovascular parameters such as ECG, pulse, blood pressure, cardiac output and stroke volume.

Repeated dose toxicity

- No haematological or histological abnormalities were observed in rats after oral administration of the extract at 4.0 g/kg/day for 20 days.
- No treatment-related haematological or histopathological findings were observed in beagle dogs after oral administration of the extract at 1.5, 5.0 and 15 mg/kg/day for 90 days.

- An additional study showed no changes in haematological parameters in rats following subcutaneous injection of a ginseng extract (corresponding to 100 mg of root) daily for 5 days [52].

Reproductive toxicity

- No decrease in growth rate or reproduction and no treatment-related haematological or histopathological findings were observed in rats during a 33-week two-generation study with the ginseng extract administered orally at 1.5, 5.0 or 15 mg/kg body weight/day.

Embryo, foetal and perinatal toxicity

- No abnormalities of foetal development were found after oral administration of the ginseng extract to rats at 40 mg/kg/day during days 1-15 after mating or to rabbits at 20 mg/kg/day during days 7-15 after mating.

Genotoxicity

- In the hepatocyte-DNA repair test no genotoxicity of the ginseng extract was observed at concentrations of 0.1-10 mg/ml, with or without ginsenosides, or of ginsenoside Rg_1 at 1-50 µg/ml.

In a study involving intraperitoneal administration of ginsenosides Rb_1 and Rg_1 to mice at 25, 50 or 100 mg/kg body weight, neither single nor repeated large doses of 100 mg/kg caused any toxic symptoms or adverse behavioural effects such as ataxia or sedation [86].

Clinical safety data

In a double-blind, placebo-controlled study involving 60 females and 60 males, daily oral administration of 200 mg of a standardized ginseng extract for 12 weeks did not cause any significant differences in blood levels of sex hormones (luteinizing hormone, follicle-stimulating hormone, testosterone, oestradiol) in comparison with placebo groups [25].

In an open study of 49 menopausal women (33 of whom had undergone a hysterectomy) regular speculum examinations and cytological smears from the cervix and vaginal wall did not reveal any changes during 3 months of oral treatment with 200 mg of a standardized ginseng extract per day [26].

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HAMAMELIDIS AQUA

Hamamelis Water

DEFINITION

Hamamelis water is a clear, colourless distillate prepared from recently cut and partially dried dormant twigs of *Hamamelis virginiana* L.

The material complies with the monograph of the United States Pharmacopeia [1].

CONSTITUENTS

From 1 kg of partially dried dormant twigs the USP method of preparation yields approximately 1 litre (980 g) of hamamelis water containing 14-15% of ethanol (added after distillation) [1]. Since it is obtained by a distillation process, the constituents are those of the volatile fraction, devoid of tannins [2].

Distillation of fresh twigs yielded 0.09% of volatile fraction on the dry weight basis, consisting of aliphatic hydrocarbons (45.4%, predominantly alkanes), terpenes (approx. 30%; mainly sesquiterpenes such as α-ylangene and monoterpenes such as linalool), phenylpropanoids (7.5%) such as *trans*-anethole and eugenol, and aliphatic aldehydes (6.1%) and alcohols (5.3%); over 160 compounds were detected [3,4].

Distillation of fresh leaves yielded 0.13% of volatile fraction on the dry weight basis consisting of aliphatic hydrocarbons (62.8%, predominantly alkanes), terpenes (21.1% including 3.7% of linalool and 9.8% of the acyclic diterpene *trans*-phytol), aliphatic aldehydes (3.8%) and fatty acids and fatty acid esters (3.6%); over 170 compounds were detected [3,4].

CLINICAL PARTICULARS

Therapeutic indications

External use

Treatment of bruises, skin irritations, sunburn, insect bites, external haemorrhoids [2,5-11]. Minor inflammatory conditions of the skin and mucosa [2,5-8].

Posology and method of administration

Dosage

External use

For compresses: Hamamelis water undiluted or diluted 1:3 with water; in semi-solid preparations, 20-30% [2,6-12]. Apply as often as required [5].

For mucosa: Hamamelis water undiluted or diluted with water, several times daily [2].

Method of administration
For local application.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available.

Effects on ability to drive and use machines
None known.

Undesirable effects
Although the content of volatile fraction is very low, allergic reactions of the skin may occur in very rare cases [2,5].

Overdose No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
In the following text, material prepared by distillation of partially dried dormant twigs of *Hamamelis virginiana* and complying with the monograph for Witch Hazel USP [1] (or Hamamelis Water BPC 1973 [13], which is essentially similar) is described as 'hamamelis water'. Material prepared by distillation of fresh leaves and twigs of *Hamamelis virginiana* is described as 'hamamelis distillate'.

Pharmacological studies in humans

Anti-inflammatory effect
The activity of hamamelis distillate against erythema was evaluated in creams with two concentrations of the drug (standardized to 0.64 mg or 2.56 mg of hamamelis ketones per 100 g) and two different vehicles: oil-in-water emulsions, with or without phosphatidylcholine (PC). The effects were compared with those of hydrocortisone 1% cream, four base preparations and an untreated control area in two randomized, double-blind studies, each involving 24 healthy volunteers; in one study erythema was induced by UV irradiation, in the other by repeated stripping of the horny layer with adhesive tape. 24 hours after UV-irradiation noteworthy reductions in erythema were observed only after use of low dose hamamelis-PC cream and hydrocortisone 1% cream, more pronounced from the latter. Erythema 4-8 hours after stripping of the horny layer was significantly suppressed by hydrocortisone cream, while less pronounced but noteworthy reductions were observed with low and high dose hamamelis-PC creams. The results demonstrated some anti-inflammatory activity of hamamelis distillate in a PC-containing vehicle. A four-fold increase of drug concentration did not, however, increase activity [6].

The anti-inflammatory effects of an aftersun lotion containing 10% of hamamelis water, in comparison with the corresponding vehicle, were tested in 30 healthy volunteers using a UV-B erythema test at four different UV-B intensities. Chromametry was used to compare the degrees of erythema in treated areas of skin with those in irradiated but untreated control areas 7, 24 and 48 hours after irradiation. Erythema suppression ranged from approx. 20% at 7 hours to 27% after 48 hours in the hamamelis water-treated areas, and from 10% at 7 hours to 12% after 48 hours in areas treated with the vehicle. Hamamelis water led to a highly significant reduction in erythema compared to the vehicle ($p = 0.00001$) and untreated, irradiated skin ($p = 0.00001$) [8].

Hamamelis distillate ointment applied to the skin of 22 healthy volunteers, and also 5 patients suffering from atopic neurodermitis and psoriasis, had a mild anti-inflammatory effect, causing a decrease in blood circulation as indicated by measurements of the thermal conductivity of the skin (fluvography) [7].

Clinical studies

Analgesic effect
In a randomised, open study involving 300 postnatal mothers, three topical agents were evaluated for their efficacy in achieving analgesia for episiotomy pain following instrumental (forceps) vaginal delivery: hamamelis water, or ice or a foam containing hydrocortisone acetate 1% and pramoxine hydrochloride 1%. Oral analgesics were permitted and taken to the same extent in all three groups. According to data collected from 266 women, the three topical agents were equally effective in achieving analgesia, with no significant differences on day 1, although from subjective and professional assessment about one-third of all mothers derived little benefit from any agent. On days 3 and 5 ice tended to be better. 126 mothers were further assessed after 6 weeks; no differences were found between the three groups in terms of healing, pain and intercourse patterns [12].

Dermatological conditions
In a randomized, double-blind study, 22 patients suffering from atopic dermatitis were treated on one

forearm with an ointment containing hamamelis distillate (25 g/100 g) and on the other forearm with bufexamac ointment (50 mg/g) three times daily for 3 weeks. From assessment of symptoms such as reddening, scaling, lichenification, itching and infiltration no statistical difference was observed between the two treatments; both forearms showed clear improvements [9].

In a randomized, double-blind, paired trial, 72 patients suffering from moderately severe atopic eczema were treated for 2 weeks with a cream containing hamamelis distillate (5.35 g/100g), or the corresponding vehicle-only cream, or hydrocortisone 0.5% cream. The reduction in total scores for three basic criteria, itching, erythema and scaling, was significantly greater ($p<0.0001$) following application of hydrocortisone in comparison with hamamelis distillate, while the score for the hamamelis preparation did not differ from that of the vehicle [10].

In another double-blind study, 116 patients with eczema of different aetiologies were treated with either ointment A containing hamamelis distillate (25 g/100g) or hamamelis preparation B (not defined, but presumed to contain distillate) as a control, or both preparations (applied to different hands), several times daily for 4-6 weeks. Improvements in the symptoms of itching, burning sensations, infiltration, reddening and scaling were observed in the majority of cases with both preparations; ointment A gave superior results with endogenous eczema but not with toxic-degenerative eczema [11].

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HAMAMELIDIS CORTEX

Hamamelis Bark

DEFINITION

Hamamelis bark consists of the dried bark from stems and branches of *Hamamelis virginiana* L., collected in spring. It contains not less than 4.0% of hide powder-precipitable tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) and calculated with reference to the dried drug.

The material complies with the monograph of the Deutscher Arzneimittel-Codex [1] or the British Herbal Pharmacopoeia [2].

CONSTITUENTS

The main characteristic constituent is hamamelitannin, a mixture of the α- and β-forms of 2',5-di-O-galloyl-hamamelose [1-8]. Proanthocyanidins are also present including: procyanidin dimers such as catechin-(4α→8)-catechin, 3-O-galloyl-epicatechin-(4β→8)-catechin [6-9] and epicatechin-(4β→8)-catechin-3-O-(4-hydroxy)benzoate; prodelphinidins such as epigallocatechin-(4β→8)-catechin, 3-O-galloyl epigallocatechin-(4β→8)-catechin and 3-O-galloyl epigallocatechin-(4β→8)-gallocatechin [9]; and proanthocyanidin oligomers consisting of 4-9 catechin/gallocatechin units, some of which are 3-O-galloylated [9-11].

Other constituents include flavan-3-ols such as (+)-catechin, (+)-gallocatechin, (–)-epicatechin-3-O-gallate, and (–)-epigallocatechin-3-O-gallate [6,8]; di-and tri-O-galloyl-hamameloses and related 4-hydroxybenzoates [5,8,9], pentagalloyl glucose [6], gallic acid [6,7] and about 0.1% of volatile oil [3].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Inflammation of mucous membranes of the oral cavity [2-3,12,13].

Short-term symptomatic treatment of diarrhoea [2,13].

External use

Haemorrhoids [2-3,12-16], minor injuries and local inflammations of the skin [3,12,13,16].

Symptomatic treatment of problems related to varicose veins, such as painful and heavy legs [3,12,15].

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Posology and method of administration

Dosage

Internal use
2-10 g of the drug daily as a decoction, used as a mouthwash [3,12,15], or 2-3 g daily as a tea [12].
2-4 ml of tincture, used diluted as a mouthwash 3 times daily [2].
Other preparations: the equivalent of 0.1-1 g of the drug, 1-3 times daily [3,12].

External use
5-10 g of the drug as a decoction in 250 ml of water [3].
Extracts in semi-solid or liquid preparations corresponding to 20-30% of the drug [3].

Method of administration
For oral administration or local application.

Duration of administration
No restriction. Medical advice should be sought if diarrhoea persists for more than 4 days.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used internally during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None known from topical application [17]. In sensitive persons, stomach irritation may occasionally occur after intake of hamamelis bark preparations [3].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Astringent effect
The astringent effect of a tincture (1:3; 62% ethanol) prepared from fresh hamamelis bark was demonstrated with hide powder [18].

Cytotoxic activity
After 4 days of incubation, polyphenols isolated from hamamelis stem and twig bark showed moderate cytotoxicity to GLC_4 lung carcinoma and COLO 320 cells. The 3-O-galloyl compounds were more effective than other compounds. IC_{50} values of galloyl compounds were between 38 µM and 110 µM for GLC_4 and between 18.3 µM and 90.8 µM for COLO 320 cells; almost complete inhibition of growth was observed at 200 µM [8].

Anti-inflammatory effects
In the lyso-PAF:acetyl-CoA acetyltransferase assay, hamamelitannin proved to be ineffective [10], but in the same assay a proanthocyanidin oligomer isolated from hamamelis bark showed inhibitory potential [8,10]. A range of compounds from hamamelis bark had an inhibitory effect on 5-lipoxygenase (from a cytosol fraction of RBL-1 cells), galloyl compounds showing greater potency than other substances; hamamelitannin had the strongest effect with an IC_{50} of 1.0 µM [8,10].

Anti-inflammatory effects of polyphenols isolated from hamamelis stem and twig bark were evaluated in human polymorphonucleocytes (PMNs) and human macrophages. With the exception of hamamelitannin, all the tested substances inhibited the synthesis of platelet activating factor (PAF) in human PMNs. Dimeric galloylated proanthocyanidins showed the strongest effects with IC_{50} values of 7.8 and 6.4 µM. The synthesis of leukotriene B_4 (LTB_4) in PMNs was inhibited by the tested substances. Oligomeric proanthocyanidins had stronger activity (IC_{50}: 1.5 µM) than hamamelitannin, which had the weakest effect (IC_{50}: 12.5 µM). The polyphenols were shown to inhibit zymosan-induced luminol-dependent chemiluminescence in human macrophages, with galloylated proanthocyanidins having stronger effects (IC_{50}: 2.3 and 2.0 µM) than hamamelitannin (IC_{50}: 10.5 µM) [8].

Antiviral activity
Hamamelitannin and fractions obtained by ultrafiltration from a hydroethanolic extract of hamamelis bark exhibited antiviral activity against *Herpes simplex* virus type 1 (HSV-1) in monkey kidney cells. After 2-3 days the ED_{50} of hamamelitannin for antiviral activity was 26 µg/ml, compared to 6.3 µg/ml for a fraction consisting mainly of oligomeric to polymeric proanthocyanidins and 0.42 µmol/ml for acyclovir as a positive control [19].

Radical-scavenging effects
A dry 50%-ethanolic extract from hamamelis bark exhibited active-oxygen scavenging activity, determined by an electron spin resonance (ESR) spin-

trapping technique, with IC_{50} values of 0.17 µg/ml for superoxide anions, 7.79 µg/ml for hydroxyl radicals and 44.08 µg/ml for singlet oxygens, compared to 4.10, 3.30 and 21.18 µg/ml respectively for ascorbic acid. The extract at 50 µg/ml also protected murine dermal fibroblasts from cell damage induced by active-oxygen, increasing the survival rate to 69.0% ($p<0.01$) compared to about 15% for the control [20].

A suppressive effect of hamamelitannin against depolymerization of hyaluronic acid (induced by a xanthine/xanthine oxidase system) was demonstrated by measuring the viscosity of a 0.9 mg/ml solution; the inhibitory rate was 73.8% for hamamelitannin compared to 24.7% for ascorbic acid and 84.4% for superoxide dismutase [21].

The radical scavenging properties of hamamelitannin and gallic acid were evaluated in further experiments using ESR spin-trapping. For superoxide anion scavenging, the IC_{50} values were 1.31 µM for hamamelitannin and 1.01 µM for gallic acid, compared to 23.31 µM for ascorbic acid [21-23]. In hydroxyl radical scavenging, hamamelitannin gave the lowest IC_{50} of 5.46 µM, compared to 78.04 µM for gallic acid and 86.46 µM for propyl gallate (a well-known anti-oxidant). In singlet oxygen scavenging, the IC_{50} values of hamamelitannin and gallic acid were 45.51 µM and 69.81 µM respectively, compared to 66.66 µM for propyl gallate [22].

Hamamelitannin was also found to have antioxidative and scavenging activities against organic radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH). Expressed as an index number (the number of mol required to scavenge one mol of DPPH), hamamelitannin and gallic acid gave results of 9.4 and 8.8 respectively, compared to 2.2 for DL-α-tocopherol and 2.0 for ascorbic acid [22].

The protective activities of hamamelitannin and gallic acid on cell damage induced by superoxide anion radicals, were evaluated in a cell-culture system using murine fibroblasts. Hamamelitannin and gallic acid showed significant protective activity against superoxide radicals at minimum concentrations of 50 µM and 100 µM respectively ($p<0.01$) [22,23]; at 50 µM, hamamelitannin enhanced the survival of fibroblasts to 52.4% compared to 36.9% for the control [23]. Pre-treatment of fibroblasts with hamamelitannin at 200 µM for 24 hours at 37°C before exposure to superoxide anions increased cell survival to 63.8%, compared to 25.4% for gallic acid and 19.0% for the control. Further observations confirmed that hamamelitannin is superior to gallic acid in protecting against cell damage induced by superoxide anions and suggested that the high affinity of hamamelitannin for cells or membranes may be an important factor for protecting cells against active oxygen species [23].

In contrast, against cell damage induced in murine fibroblasts by hydroxyl radicals, hamamelitannin showed protective activity at a minimum concentration of 500 µM whereas gallic acid was effective at 50 µM. Against cell damage induced by singlet oxygens hamamelitannin at 100 µM enhanced survival to 80.6% ($p<0.01$), while gallic acid had no significant effect at 100 µM and required 500 µM to enhance survival to 98.6% ($p<0.01$) compared to 60.4% survival for controls [22].

Hamamelitannin and a fraction of molecular weight < 3 kDa obtained by ultrafiltration from a hydro-ethanolic hamamelis bark extract were found to have greater radical scavenging activity (ED_{50} values of 29 and 80 ng/ml respectively) than a higher molecular weight procyanidin fraction (≥ 3 kDa; ED_{50} 160 ng/ml) as quantified by the emission of chemiluminescence during autoxidation of mouse brain lipids [19].

Antimutagenic activity

In the Ames mutagenicity test, a tincture (1:5) and a methanolic extract (1:5) of hamamelis bark dose-dependently inhibited 2-nitrofluorene-induced mutagenicity in *Salmonella typhimurium* TA98, by 60% and 54% respectively at 100 µl/plate. It was demonstrated that the antimutagenic effect increased with increasing degree of polymerisation of pro-anthocyanidins, the most active fraction consisting of catechin and gallocatechin oligomers with an average degree of polymerization of 9.2 [11].

In vivo experiments

Anti-inflammatory effect

A hydroethanolic extract of hamamelis bark showed a significant anti-inflammatory effect (43% inhibition of oedema; $p<0.05$) in the croton oil ear oedema test in mice when applied topically at 250 µg per ear. After ultrafiltration of the crude extract, this effect was shown to be mainly due to proanthocyanidins of molecular weight ≥ 3 kDa (69% inhibition at 250 µg per ear; $p<0.05$); proanthocyanidins of lower molecular weight had no effect and hamamelitannin produced only 7% inhibition [19].

Clinical studies

In a double-blind, three-arm comparative study, 90 patients with first-degree haemorrhoids were treated for 14-21 days with an ointment containing hamamelis bark fluid extract (3% m/m) and basic bismuth gallate (n = 30), or metacresolsulphonic acid-formaldehyde ointment (n = 30) or ointment containing the corticosteroid fluocinolone acetonide (n = 30); all three preparations also contained a local anaesthetic. Follow-up examinations were performed on days 3, 7, 14 and 21 of treatment. At the end of treatment, the levels of improvement in four target criteria (pruritus, bleeding, burning sensation and pain) assessed by both physicians and patients were 73.9-94.4% in

patients using the ointment containing hamamelis bark, compared to 76.2-89.5% in those treated with metacresol-sulphonic acid-formaldehyde and 72.0-81.8% in those treated with fluocinolone acetonide. All three preparations were considered highly effective with no major differences between groups [14].

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HAMAMELIDIS FOLIUM

Hamamelis Leaf

DEFINITION

Hamamelis leaf consists of the whole or cut, dried leaf of *Hamamelis virginiana* L. It contains not less than 3 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

The main characteristic constituents are tannins (5-10%) [2], including condensed tannins (mainly proanthocyanidin oligomers with catechin and/or gallocatechin units) and hydrolysable gallotannins, notably a small amount of hamamelitannin [3-5]. (+)-Catechin, (+)-gallocatechin, (–)-epicatechin-gallate and (–)-epigallocatechingallate are also present [6].

Other constituents include flavonoids such as kaempferol, quercetin, quercitrin, isoquercitrin and myricetin; phenolic acids such as caffeic acid and gallic acid [3,7-9]; and a volatile fraction, 0.04-0.14% [10], containing aliphatic hydrocarbons (63%), mono- and sesquiterpenes (11%) and aldehydes and ketones (4.6%) among over 170 compounds detected [11].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Symptomatic treatment of complaints related to varicose veins, such as painful and heavy legs, and of haemorrhoids [7-9,12,13].

External use

Bruises, sprains and minor injuries of the skin [7,8,14].
Local inflammations of the skin and mucosa [7-9].
Haemorrhoids [7,8,15].
Relief of the symptoms of neurodermitis atopica [16] and feeling of heavy legs [7].

Posology and method of administration

Dosage

Internal use

Adults: 2-3 g of drug as infusion [7] or 2-4 ml of liquid extract (1:1, 45% ethanol), three times daily [15].

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External use
Extracts in semisolid or liquid preparations, containing 5-10% of drug [7].
Decoctions, 5-10 g of drug per 250 ml water for compresses or washes [7].
Suppositories containing 200 mg of dried extract, 1-2 per day [14].
Ointment containing 10% of liquid extract [14].

Method of administration
For oral administration and topical application.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the drug should not be used internally during pregnancy without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In sensitive patients there is a possibility of stomach upsets after taking hamamelis leaf preparations [7].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vivo experiments
Venotonic activity was demonstrated in experiments where the dried residue (300 mg) from aqueous or various hydroethanolic extracts of hamamelis leaf was added to one litre of an isotonic dextran/water (60 g/litre) solution. This was perfused at constant pressure into the arteries of the hind quarters of rabbits (45-100 drops/min). Venoconstriction, measured in terms of output on the venous side, was reduced by up to 60-70% depending on the type of extract [17].

A dry 70%-ethanolic extract of hamamelis leaf, administered orally to rats at 200 mg/kg daily for 19 days, significantly inhibited paw swelling ($p<0.05$) in the chronic phase of adjuvant-induced arthritis but was not active against the acute phase of oedema [18].

Pharmacological studies in humans
In a study conducted on 30 human volunteers, topical application of a hydroglycolic extract of hamamelis leaf produced a significant reduction in skin temperature ($p<0.001$ after 5 min; $p<0.03$ after 60 min), which was interpreted as a vasoconstrictor effect [19].

Clinical studies
In a pilot study involving cases of neurodermitis atopica, hamamelis leaf extract incorporated into a cream was applied twice daily for 2 weeks in six groups of patients:
- Group I consisted of 7 children aged from 6 to14 years with atopic neurodermitis on the feet (chilblains). After treatment the condition had considerably improved in all the patients.
- Group II consisted of 5 children with eczema in the flexure of the joints in subacute and chronic forms. After treatment 3 children were completely cured; in 2 children a considerable reduction of the inflamed condition and a clear reduction of the itch was noted.
- In Group III, which consisted of 10 adults with eczema in the flexure of the joints, 7 cases showed a good response and in three cases there was reasonable improvement.
- Group IV consisted of 5 adults with eczema of the neck and throat. 3 cases were completely cured and a remarkable reduction in symptoms was noted in the remaining 2 cases.
- Group V consisted of 3 adults with atopic eczema of the trunk. In one patient there was extensive healing and in the others a noticeable reduction of symptoms.
- Group VI consisted of 2 cases of atopic xerodermia. Following twice daily application of the cream, there was an improvement of the skin barrier situation and a clear diminution of desquamating pruritus in both cases [16].

Pharmacokinetic properties
No data available.

Preclinical safety data

Carcinogenicity
15 male and 15 female NIH black rats, 1-2 months old, were injected subcutaneously with a lyophilised aqueous leaf extract at a dosage of 10 mg (dissolved in 0.5 ml of normal saline solution) weekly for a period of 78 weeks; 30 animals were injected with normal saline solution only. The animals were observed for a period of 90 weeks. After about 73 weeks, three of the male rats developed malignant mesenchymoma, while in the control animals no tumours developed. The tumour rate was not considered to be significant [7].

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HARPAGOPHYTI RADIX

Devil's Claw Root

DEFINITION

Devil's claw root consists of the cut and dried tuberous, secondary roots of *Harpagophytum procumbens* D.C. It contains not less than 1.2 per cent of harpagoside ($C_{24}H_{30}O_{11}$; M_r 494.5), calculated with reference to the dried drug [1].

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

The characteristic constituents are iridoid glucosides, principally harpagoside (1-3%) [2-5] together with small amounts of harpagide, 8-*p*-coumaroylharpagide (0.03-0.13%), procumbide and its 6'-*p*-coumaroyl ester [3-7]. The phenolic glycosides acteoside (verbascoside) and isoacteoside [8], and sugars, mainly the tetrasaccharide stachyose (up to 46%) with smaller amounts of raffinose, sucrose and monosaccharides [9], are also present.

CLINICAL PARTICULARS

Therapeutic indications

Symptomatic treatment of painful osteoarthritis [10-18], relief of low back pain [19-23], loss of appetite and dyspepsia [24-30].

Posology and method of administration

Dosage

Painful osteoarthritis

Adult daily dose: 2-5 g of the drug or equivalent aqueous or hydroalcoholic extracts [11,13-15,17, 18].

Relief of low back pain

Adult daily dose: 4.5-9 g of the drug as dry extract equivalent to 30-100 mg of harpagoside [19-23].

Loss of appetite or dyspeptic complaints

Adult dose: 0.5 g of the drug in decoction, three times daily, or preparations with equivalent bitterness value [10,24]; tincture (1:10, 25% ethanol), 3 ml [10,25].

Elderly: dose as for adults.
Not recommended for children.

Method of administration

For oral administration.

Duration of administration
Treatment for at least 2-3 months is recommended in cases of painful osteoarthritis [11,14,16]. If symptoms persist consult a doctor.

Contra-indications
Gastric and duodenal ulcers [24,30].

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Mild gastro-intestinal disturbances (e.g. diarrhoea, nausea, stomach upset) may occur in sensitive individuals especially at higher dosage levels [14,16,19].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

The anti-inflammatory, analgesic, antiarrhythmic and hypotensive effects of devil's claw root and the iridoid glucoside harpagoside have been extensively investigated.

In vitro experiments
A crude methanolic extract of devil's claw root (1 mg and 2 mg, containing 0.085 mg and 0.17 mg of harpagoside respectively) and, to a lesser extent, pure harpagoside (0.085 mg and 0.17 mg) showed a significant, dose-dependent, protective action against arrhythmias induced by reperfusion in isolated rat hearts [31]. The same methanolic extract (1 mg) showed a protective effect against arrhythmias induced by calcium chloride and epinephrine-chloroform in isolated rabbit heart [32].

Following oral administration to 6 volunteers of 600 mg of devil's claw root extract containing ca. 25% harpagoside, the effect on biosynthesis of eicosanoids was studied *ex vivo* in samples of their blood. After stimulation with ionophore A23187 for 60 minutes, the synthesis of thromboxane B_2 (an indicator of the cyclooxygenase metabolic pathway) and leukotriene C_4 (an indicator of the 5-lipoxygenase metabolic pathway) were measured by radio-immunoassay. Blood samples of all subjects revealed a time-dependent reversible inhibition of leukotriene C_4 biosynthesis with maximum inhibition of 50% after ca. 3 hours. The biosynthesis of thromboxane B_2 was not inhibited [33,34].

Human whole blood samples from healthy young males were pretreated with devil's claw root extracts containing 7.3% and 2.07% of harpagoside respectively, or pure harpagoside or BAY X1005 (a synthetic 5-lipoxygenase inhibitor used as a reference substance), stimulated with ionophore A23187 and incubated, then analyzed to compare their effects on the biosynthesis of cysteinyl leukotrienes (metabolites of the 5-lipoxygenase metabolic pathway) and thromboxane B_2 (metabolite of the cyclooxygenase metabolic pathway). BAY X1005 or harpagoside at 1-100 µM concentration-dependently inhibited the biosynthesis of cysteinyl leukotrienes with IC_{50} values of ca. 6.5 µM and 39.0 µM respectively. The devil's claw root extracts showed similar inhibition in proportion to their harpagoside contents (7.3% and 2.07%), with calculated harpagoside IC_{50} values of 9.2 µM and 61.7 µM respectively. Thus the extract with a harpagoside content of 7.3% produced more effective inhibition than pure harpagoside. With respect to the biosynthesis of thromboxane B_2 BAY X1005 did not show significant inhibition ($IC_{50} > 100$ µM); harpagoside and devil's claw root extract (7.3% harpagoside) gave harpagoside IC_{50} values of 48.6 µM and 55.3 µM respectively, while devil's claw root extract (2.07% harpagoside) gave a harpagoside IC_{50} of > 100 µM. An explanation of the observed effects could be that harpagoside effectively inhibits the biosynthesis of both cysteinyl leukotrienes and thromboxane B_2 in eicosanoid metabolic processes, but that devil's claw root extracts contain other substances which may, directly or indirectly, inhibit the biosynthesis of cysteinyl leukotrienes [34,35].

An endotoxin-free dry extract of devil's claw root, prepared with ethanol 60% V/V and containing 2.9% harpagoside, prevented lipopolysaccharide (LPS)-induced synthesis of tumour necrosis factor alpha (TNF-α) in stimulated primary human monocytes in a dose-dependent manner with an IC_{50} of about 100 µg/ml. Harpagoside and harpagide had no effect on LPS-induced TNF-α release between 0.01 µg/ml and 10 µg/ml [36].

In vivo experiments

Anti-inflammatory effects
In repeated dose studies (formaldehyde-, Freund adjuvant- and granuloma-induced experimental arthritis), extracts of devil's claw root appeared to be effective [37,38] although other studies have not confirmed these results [39-41]. In the croton oil-

induced granuloma pouch test in rats the reduction in inflammation produced by 12-day intraperitoneal administration of harpagoside (20 mg/kg/day) [37] and by oral administration of aqueous and methanolic extracts of devil's claw root (200 mg/kg/day) [38] was similar to that of phenylbutazone. In the formaldehyde-induced arthritis test an effect comparable to that of phenylbutazone (40 mg/kg/day) was demonstrated with an aqueous extract of devil's claw root (20 mg/kg/day) after 10-day intraperitoneal administration, but no effect was apparent with harpagoside (50 mg/kg/day) [37]. Daily oral treatment with devil's claw root at a high dose level of 2 g/kg for 7 days produced no significant effect on secondary inflammatory reaction in the rat [41]. Adjuvant (*M. tuberculosis*)-induced arthritis was also unresponsive to treatment with a dry aqueous extract of devil's claw root (100 mg/kg/day) administered orally for 21 days in the rat [39].

No, or only slight, activity of devil's claw root or harpagoside in acute conditions (carrageenan-induced oedema etc.) has been found by several authors [37-42]. On the other hand, a 48% reduction in adriamycin-induced oedema in rats was obtained after oral administration of 37 mg/kg of powdered root containing 3.0% of iridoid glucosides [43].

Intraperitoneal pre-treatment of rats with a dry aqueous extract (2.2% harpagoside) of devil's claw root significantly reduced carrageenan-induced hind paw oedema, in a dose-dependent manner. Doses of 400 and 1200 mg/kg, corresponding to 665 and 2000 mg/kg of dried root, reduced oedema 3 hours after administration by 43% and 64% respectively. The efficacy of the 1200 mg/kg dose was similar to that of indometacin 10 mg/kg [4]. In the same model, a dry ethanolic extract (400 mg/kg) had an anti-inflammatory effect (68.8% inhibition) similar to that of phenylbutazone (150 mg/kg) 4 hours after oedema induction [44]. Significant, dose-dependent, anti-inflammatory effects in the carrageenan-induced paw oedema test have also been demonstrated following intraperitoneal pre-treatment of rats with devil's claw root aqueous extract with a harpagoside content of 1.8% at dose levels of 100 mg/kg (38% inhibition) to 400 mg/kg (72% inhibition). The highest dose tested (400 mg/kg) was more effective than pre-treatment with 10 mg/kg of indometacin (58% inhibition). Pure harpagoside was ineffective in these experiments [45].

Another study using the carrageenan-induced rat paw oedema test assessed the anti-inflammatory activity of devil's claw root extracts when administered by different routes: dry aqueous extracts, prepared by lyophilization from cryoground fresh plant, without cyclodextrin (intraperitoneal administration) or with cyclodextrin (oral and intraduodenal administration). The results indicated that intraperitoneal pre-treatment of rats with doses of 200 and 400 mg of extract significantly reduced carrageenan-induced oedema ($p<0.001$). Similarly, intraduodenal pre-treatment with 200, 400 and 1600 mg of extract significantly reduced oedema ($p<0.01$). In contrast, when administered orally (by gavage), the extracts were ineffective regardless of the dose used (200, 400, 800 and 1600 mg/kg) [46]. This is consistent with the results of an earlier study [45], which showed absence of activity after treating the extract with 0.1 N hydrochloric acid, simulating acid conditions in the stomach.

Since these results support the inference that gastric degradation of active principles may occur, the use of oral preparations protected against degradation by stomach acid has been suggested [45,46].

Analgesic effects

A devil's claw root aqueous extract with a harpagoside content of 1.8% exhibited dose-dependent peripheral analgesic effects in the writhing test after intraperitoneal administration to mice (47% protection at 100 mg/kg to 78% at 400 mg/kg). 53% protection at 200 mg/kg was similar to the 59% result obtained with acetylsalicylic acid at 68 mg/kg; pure harpagoside at 10 mg/kg produced 42% protection [45]. In earlier work using the rabbit ear test, intraperitoneal administration of harpagoside (20 mg/kg) produced an analgesic effect comparable to that of phenylbutazone (50 mg/kg), but harpagoside (20 mg/kg) hydrolysed to its aglycone by emulsin and an aqueous extract of devil's claw root (20 mg/kg) showed no statistically significant effects [37]. The number of writhings and stretchings induced in rats by 1.2% acetic acid was significantly and dose-dependently reduced after intraperitoneal administration of devil's claw root dry aqueous extract (2.2% harpagoside); the protective effect was 35% at 400 mg/kg and reached 62% at 1200 mg/kg, compared to 59% with acetylsalicylic acid at 68 mg/kg [4].

In a Randall-Soletto test, the threshold of pain induced in rats by subplantar injection of 0.1 ml of 20% yeast solution was measured just before, 30 minutes after and 60 minutes after intraperitoneal administration of a devil's claw root dry ethanolic extract of undefined potency. 200 and 400 mg/kg of extract dose-dependently increased the pain threshold, the effect after 30 minutes (28.5% and 61.5% respectively) being greater than that after 60 minutes; 800 mg/kg produced no further increase. The effects were superior to those of diclofenac sodium at 80 mg/kg (11.1% increase after 30 minutes) [44].

Other workers found no consistent analgesic effects in mice after oral administration of various extracts and fractions from devil's claw root at 20 and 200 mg/kg [38].

Other effects

A dry 53%-ethanolic extract of devil's claw root was

administered intraperitoneally to rats at 100 mg/kg and 200 mg/kg bodyweight daily for 1, 7 or 14 days to investigate its antioxidant activity in comparison with selegiline (2 mg/kg). The extract induced an increase in brain frontal cortex and striatum superoxide dismutase, catalase and glutathione peroxidase activities, and decreased lipid peroxidation, in a dose-related manner. The effects, evident only after 7 days of treatment and accentuated after 14 days, were similar to responses induced by selegiline [47].

Both oral and intraperitoneal treatment of rats with a dry methanolic extract of devil's claw root containing 8.5% of harpagoside gave considerable protection against arrhythmias induced by calcium chloride or epinephrine-chloroform. An oral dose of 400 mg/kg of extract produced 50% more effective protection against calcium chloride-induced arrhythmia than an oral dose of 100 mg/kg of lidocaine. Pure harpagoside gave much weaker protection than extract containing equivalent amounts of harpagoside [32].

Pharmacological studies in humans

No significant effects on mediators of acute inflammation (prostaglandin E_2, thromboxane B_2, 6-ketoprostaglandin $F_1\alpha$ and leukotriene B_4) were evident in 25 healthy volunteers after a 3-week daily intake of 4 × 500 mg capsules of powdered devil's claw root containing 3% of iridoid glucosides. The subjects served as their own control and were also compared with a separate control group. It was concluded that devil's claw root does not produce the biochemical effects on arachidonic acid metabolism characteristic of anti-arthritic drugs of the non-steroidal anti-inflammatory type [48].

Clinical studies

Relief of arthrosic and arthritic conditions

In a double-blind, placebo-controlled study on ambulant volunteers with articular pains of rheumatic origin, the efficacy and tolerability of capsules containing 335 mg of powdered devil's claw root (3.0% iridoid glucosides) were assessed at a dosage of 3 × 2 capsules daily for 2 months. Clinical parameters measured on days 0, 30 and 60, severity of pain on a 0-10 scale and joint mobility determined by finger-to-floor distance during anteflexion of the trunk, revealed a significant drop in the intensity of pain ($p<0.05$) and a significant increase in spinal and coxofemoral mobility ($p<0.05$) in the verum group (n = 45) compared to the placebo group (n = 44) after 30 and 60 days. Neither side effects nor changes in biological parameters (including blood tests) were observed during the study [11].

In a double-blind, placebo-controlled study, 50 volunteers suffering from arthrosis were given 3-week courses of daily treatment with 3 × 2 capsules, each containing 400 mg of devil's claw root hydroethanolic extract (1.5% iridoid glucosides), or placebo. Assessments were carried out 10 days after completion of treatment, with evaluation of the severity of pain in 5 conditions on a 0-4 scale. Individual patients were given from one to three courses of treatment. Compared to placebo, the extract produced a statistically significant decrease in the severity of pain. Improvements were more frequent in moderately invaliding arthrosis than in more severe cases [12].

46 patients suffering from osteoarthritis of the hip participated in a 20-week, double-blind, placebo-controlled study as two randomized groups. Patients in one group (n = 24) were treated with 2 tablets per day containing a 60% ethanolic dry extract of devil's claw root (480 mg, drug to extract ratio 4.4-5.0:1); those in the second group received placebo tablets. Both groups also received identical, stepwise-reducing daily doses of ibuprofen: 2 × 400 mg for the first 8 weeks, 1 × 400 mg for a further 8 weeks and none in the last 4 weeks of the study. Efficacy was evaluated from osteoarthritis scores reported by the patients, using the Western Ontario and McMaster Universities Arthrosis Index (WOMAC). WOMAC scores decreased in both groups over the study period, despite the reducing dosage of ibuprofen. WOMAC sub-scores for stiffness, pain and dysfunction decreased similarly in both groups. In the final, ibuprofen-free period, an increase of 20% or less in the pain score was considered a clinically relevant response rate; 71% of devil's claw patients, but only 41% of placebo patients, fulfilled this criterion ($p = 0.04$). 52% of patients in the devil's claw group, compared to 36% in the placebo group, were able to complete the study without using rescue therapy in the ibuprofen-free period. From evaluation by both patients and physicians, the tolerability of devil's claw root extract was comparable to that of placebo in the final, mono-therapy phase. Devil's claw root extract accompanied by reducing dosages of ibuprofen therefore appeared to be a possible substitution therapy in about 70% of the patients [13].

In a 4-month randomized comparative study, the effects of powdered devil's claw root at a daily dosage of 3 × 2 capsules, providing a total of 2610 mg of root containing 57 mg of harpagoside (n = 62), were compared with those of a symptomatic slow-acting drug for osteoarthritis, diacerhein at 100 mg/day (n = 60), in patients suffering from osteoarthritis of the knee and hip. Spontaneous pain and severity of osteoarthritis evaluated by Lequesne's index were significantly improved during the course of the study and there was no difference in the efficacy of the two treatments. On completion of the study, patients taking devil's claw root were using significantly less NSAIDs or other analgesics ($p = 0.002$). The most frequently reported adverse event was diarrhoea, which occurred in 8.1% and 26.7% of devil's claw root and diacerhein patients respectively. A global

tolerance assessment by patients at the end of treatment favoured devil's claw root [14].

In a comparative study, patients suffering from articular pains of rheumatic origin (n = 40) and from gout (n = 10) were randomized into two identical groups of 25. The efficacy and tolerability of 1230 mg of devil's claw root dry aqueous extract (drug to extract ratio 2:1), divided into three oral daily doses, was compared to phenylbutazone (300 mg daily during the first four days, then 200 mg daily; tablets assumed to have the standard potency of 100 mg). After 28 days, from assessment of a range of clinical parameters including pain, stiffness and mobility, devil's claw root extract was found to have been equally as effective as, or even superior to, phenylbutazone. No adverse events were recorded in the devil's claw group whereas 6 patients taking phenylbutazone reported one or more adverse events [15].

In a large open study on 630 arthrosic cases, 42% to 85% of the patients, grouped according to the site of arthroses, showed improvements after 6 months of treatment with devil's claw root dry aqueous extract (drug to extract ratio ca. 3:1) containing 2.5% of iridoid glucosides at daily dosages of 3 g to 9 g, divided into three doses. No side effects other than mild gastro-intestinal disturbances were reported, even at the highest dosage level [16].

In a 30-day controlled pilot study involving 100 patients with varied rheumatic indications (activated arthrosis, chronic low back pain, non-articular rheumatic conditions), the pain-relieving effect of a devil's claw root dry extract (2:1; ethanol 40% V/V) at a daily dose of 2460 mg (n = 50), was compared with placebo (n = 50). Favourable effects of verum medication were clearly evident after 10 days. By the end of the study, the greatest therapeutic improvement was achieved in the verum subgroup suffering from low back pain [17].

In a drug monitoring study, 675 patients (mean age: 58.1 years) with painful osteoarthritis, spondyl-arthropathies or fibromyalgic complaints were treated daily for 8 weeks with 2 × 480 mg of a devil's claw root dry extract (4.4-5.0:1, ethanol 60% V/V). The main outcome criteria were the Clinical Global Impressions (CGI) score and reduction in a symptom severity score (from 0 = no pain to 3 = strong pain). The extent of use of non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids as comedication for the underlying disease was assessed as a secondary parameter. Marked therapeutic effects were observed during the study period, the mean time to onset of action being 13 days. Due to the chronicity and phasic pattern of the disease, treatment with devil's claw root extract was continued after the monitoring phase in 79% of patients. Efficacy assessed by CGI scores was rated good or very good in 82% of cases. The symptom score for painful motion decreased by 53% from 2.23 (indicating moderate pain) to 1.04 (indicating slight pain) after 8 weeks of treatment. Over the same period, previously prescribed comedication was successfully reduced or even discontinued in 60.3% of the 464 patients taking NSAIDs and 56% of the 50 patients taking corticosteroids. A clear improvement in quality of life was evident in so far as the devil's claw root extract was rated better than their previous antirheumatic treatment by 62.4% of patients in terms of efficacy and 80% in terms of tolerability [18].

On the other hand, no significant improvements were reported in 13 patients, suffering mainly from rheumatoid arthritis, after 6 weeks of daily treatment with 3 × 410 mg of devil's claw root dry aqueous extract [40].

Relief of back pain

Daily doses of 600 mg (n = 65) or 1200 mg (n = 66) of devil's claw root dry extract (6-9:1), providing 51 mg and 102 mg of harpagoside respectively, were compared to placebo (n = 66) in a 4-week randomized, double-blind study in patients suffering for longer than 6 months from chronic low back pain (worse than 5 on a 0-10 visual analogue scale) not readily attributable to identifiable causes. 3 patients in the placebo group, 6 in the devil's claw 600 mg group and 10 in the devil's claw 1200 mg group were pain-free without rescue medication (tramadol) for at least 5 days in the last week of treatment ($p = 0.027$). Subsidiary analyses of pain experienced by individual patients suggested a significant effect of devil's claw root extract only in patients with non-radiating and less severe pain. The only side effects related to devil's claw root were infrequent and mild gastro-intestinal disturbances [19].

In a randomized, double-blind, placebo-controlled study, 118 patients with chronic low back pain were treated daily for 4 weeks with 3 × 800 mg of a devil's claw root dry aqueous extract (2.5:1) providing 50 mg of harpagoside, or placebo. At the end of treatment, 9 out of 54 patients in the verum group and 1 out of 55 in the placebo group were pain-free ($p = 0.008$). Improvement of the overall Arhus low back pain index was 20% in the verum group compared to 8% in the placebo group ($p<0.059$); although this was not statistically significant, the pain index component of the Arhus index was significant ($p = 0.016$). However, the principal outcome measure, use of supplementary analgesic (tramadol) during the final 3 weeks, did not differ between the two groups. Only minor and non-specific adverse effects occurred during treatment with devil's claw root extract [20].

In a randomized, double-blind, placebo-controlled study, 63 patients with slight to moderate muscular tension or slight muscular pain of the back, shoulder and neck received 960 mg of devil's claw root dry

extract (4.4-5.0:1, ethanol 60 % V/V), or placebo, for 4 weeks. The efficacy of verum treatment was clear from the clinical global score and patient and physician ratings. Significant effects were observed in the visual analogue scale, pressure algometer test, muscle stiffness test and muscular ischaemia test, but there were no differences from placebo in antinociceptive muscular reflexes or electromyogram activity. No serious adverse events occurred. The results imply that devil's claw root may have a beneficial effect on sensory and vascular muscle response. No evidence of CNS effects was observed [21].

In an open prospective study, 102 patients suffering from acute non-pseudoradiating low back pain for more than 6 months received either 3 × 600 mg/day of a devil's claw root dry aqueous extract (2.5:1) providing 30 mg/day of harpagoside, as a mono-therapy or combined with other therapies if needed (group J: n = 51), or conventional therapy only, mainly oral non-steroidal anti-inflammatory drugs (NSAIDs), physical exercises or paravertebral injections (group K: n = 51). The number of pain-free patients after 4 and 6 weeks of treatment was comparable between the groups (group J: 16 and 20; group K: 12 and 23, respectively). After 6 weeks of therapy, the Arhus low back pain index had improved in both groups by about 20%; the relative change in single components of the index (pain, invalidity and physical impairment) did not differ between the groups. The subgroup (n = 17) of group J receiving devil's claw root extract as mono-therapy showed improvement similar to that of groups J and K [22].

In an 8-week open study, 130 patients suffering from non-radiating chronic back pain for at least 6 months were treated daily with 960 mg of devil's claw root dry extract (4.4-5.0:1, ethanol 60% V/V). Rescue medication in the form of paracetamol was available for the first 4 weeks. Subjective perceptions of pain, evaluated by validated pain scales (the Multi-dimensional pain scale and the Arhus back pain index), decreased significantly ($p<0.001$) during treatment. The mobility of the spinal column, determined by the average finger-to-floor distance and using Schober's sign, also improved significantly ($p<0.001$). The average score obtained using the first subscale of Clinical Global Impressions decreased from 4.6 at the start of the study to 2.9 after 8 weeks of treatment. No serious adverse effects were observed; one gastro-intestinal complaint (bloating sensation) was reported [23].

Other effects

From experience of digestive disorders in a medical practice over a 3-year period, based on subjective assessment and on evaluation of clinical and biochemical parameters, the following results were obtained using decoctions of devil's claw root (1 teaspoonful to 2 cups of water): improvement in small intestine complaints, normalization of constipation and diarrhoea, elimination of flatulence and stimulation of appetite [25].

Although pharmacological experiments in rodents [45,46] indicated that enteric coated dosage forms of devil's claw root might be necessary, clinical studies [11-23] do not support this contention. Furthermore, pure harpagoside and the harpagoside (2% and 7.3%) in two devil's claw root extracts were found to be chemically stable during incubation with simulated gastric and intestinal juices at 37°C for 90-120 minutes [33,49].

Pharmacokinetic properties

In whole human blood taken from one subject after ingestion of devil's claw root extract containing 44 mg of harpagoside, the level of harpagoside after 2 hours was found to be 15.4 ng/ml [49]. After oral administration to 6 volunteers of 600 mg of devil's claw root extract containing 25% harpagoside, maximum plasma harpagoside levels were observed after 1.3 hours (32.2 ng/ml), followed by a rapid decrease. A second peak observed after 8 hours indicated enterohepatic circulation. The elimination half-life of harpagoside was 5.6 hours [34].

The iridoid glycosides harpagoside, harpagide and 8-*p*-coumaroylharpagide from devil's claw root were transformed into the pyridine monoterpene alkaloid aucubinine B by human faecal flora and by bacteria isolated from the flora. Small amounts of aucubinine B were also obtained from these iridoid glycosides by incubation with β-glucosidase in the presence of ammonium ion [50].

Preclinical safety data

Aqueous and ethanolic extracts of devil's claw root and the isolated compounds harpagoside and harpagide have shown very low toxicity in rodents during acute and subacute tests [3,38,41].

In male and female Swiss Webster mice the acute oral LD_0 of devil's claw root was greater than 13.5 g/kg [41]. The acute intraperitoneal LD_{50} of pure constituents in mice was shown to be 1 g/kg for harpagoside and greater than 3.2 g/kg for harpagide [3].

The acute oral LD_0 and intravenous LD_0 in mice of aqueous, methanolic and butanolic dry extracts of devil's claw root were found to be greater than 4.6 g/kg and 1.0 g/kg respectively [38]. A purified extract containing 85% of harpagoside showed an acute oral LD_0 greater than 4.6 g/kg and acute intravenous LD_0 and LD_{50} of 395 mg/kg and 511 mg/kg respectively [38].

In male Wistar rats, no significant haematological or

gross pathological findings were evident following 21 days of subacute oral treatment with 7.5 g/kg of devil's claw root. No hepatotoxic effects were observed with respect to liver weight or levels of microsomal protein and six liver enzymes after 7 days of oral treatment with 2.0 g/kg [41].

Because of the lack of inhibitory effects of devil's claw root on the biosynthesis of prostanoids [41,48], it has been emphasized that adverse effects often associated with non-steroidal anti-inflammatory and glucocorticoid drugs are not to be expected, even during long-term therapy [51].

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HEDERAE HELICIS FOLIUM

Ivy Leaf

DEFINITION

Ivy leaf consists of the dried leaves of *Hedera helix* L. It contains three main saponins: hederasaponin C (hederacoside C), hederasaponin B and hederasaponin D (saponin k10), with not less than 2.5% of hederasaponin C.

The material complies with the monograph of the Pharmacopée Française [1].

Fresh material may also be processed provided that when dried it complies with the monograph of the Pharmacopée Française.

CONSTITUENTS

Triterpene saponins (2.5-6%), predominantly bisdesmosidic glycosides of hederagenin with hederasaponin C (hederacoside C) as the main saponin, and a small amount of the monodesmosidic saponin α-hederin [2,3]. Other saponins present, in decreasing order of concentration, are hederasaponins B,D,F,G,E,H and I.

Other constituents include phytosterols, polyines such as falcarinol and didehydrofalcarinol, essential oil, flavonoids and other phenolic compounds such as caffeoylquinic acids [2,3].

CLINICAL PARTICULARS

Therapeutic indications

Coughs, particularly when associated with hypersecretion of viscous mucus; as adjuvant treatment of inflammatory bronchial diseases [4-11].

Posology and method of administration

Dosage

Note: Most preparations from ivy leaf contain hydroethanolic dry extracts incorporated into ethanol-containing or ethanol-free oral liquids, or suppositories. The following recommendations are:

Daily doses expressed as the corresponding amounts of dried ivy leaf

ORAL USE

Ethanol-containing preparations

Adults: 250-420 mg [5].
Children 4-12 years: 150-210 mg [8,9].
Children 1-4 years: 50-150 mg [12].

E/S/C/O/P
MONOGRAPHS
Second Edition

Children 0-1 year: 20-50 mg [12].

Ethanol-free preparations
Adults: 300-945 mg [10,11,13].
Children 4-12 years: 200-630 mg [6,7,10,11,13,14].
Children 1-4 years: 150-300 mg [10,11,14].
Children 0-1 years: 50-200 mg [10-12].

RECTAL USE

Suppositories
Children 4-10 years: 960 mg [8].

Method of administration
For oral administration in liquid or solid dosage forms; for rectal application as suppositories.

Duration of administration
If symptoms persist or worsen medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No human data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Fresh ivy leaf and the leaf sap can cause allergic contact dermatitis [15-17]. Falcarinol and didehydrofalcarinol have been reported to be allergenic [18,19].

Overdose
Overdosage can provoke nausea, vomiting, diarrhoea and excitation [20]. In these cases a physician should be consulted immediately.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Spasmolytic activity
Saponins and phenolic compounds isolated from a 30%-ethanolic extract of ivy leaf (6:1) exhibited spasmolytic activity against acetylcholine-induced contractions of isolated guinea pig ileum. Spasmolytic activity equivalent to that of 1 mg of papaverine was exerted by 169 mg of hederacoside C, 18 mg of α-hederin and 21 mg of their aglycone, hederagenin, which was not present in the extract ($p<0.05$ for all three compounds); 7 mg of kaempferol and 18 mg of quercetin ($p<0.01$, although less than 0.01% of each of these flavonol glycosides was present in the extract); and 46 mg of 3,5-dicaffeoylquinic acid ($p<0.05$; about 0.5% present in the extract). Taking into account the amounts of such constituents present in the extract in relation to their activity, the saponins α-hederin and hederacoside C appeared to contribute most of the spasmolytic activity, with α-hederin the more prominent in this respect. Each of 5 fractions of the extract, in total representing over 90% of the original extract, had spasmolytic activity (all $p<0.05$) [21,22].

Antimicrobial activity
A saponin mixture (predominantly hederacoside C) from ivy leaf exhibited antibacterial activity against Gram-positive bacteria (*Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp.) with minimum inhibitory concentrations (MICs) of 0.3-1.25 mg/ml and against Gram-negative bacteria (*Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Escherichia coli, Proteus vulgaris*) with MICs of 1.25-5 mg/ml [23]. An ethanolic extract from ivy leaf completely inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and partially inhibited the growth of *E. coli* [24].

α-Hederin at 0.5 mg/ml exhibited antifungal activity against *Candida albicans* and the dermatophyte *Microsporum canis* (0.05 mg/ml), but hederacoside C and crude saponin mixtures from ivy leaf were ineffective against these and other dermatophytic fungi [25]. Other experiments confirmed the antifungal activity of α-hederin (MIC: 0.25 mg/ml) [26].

Hederacoside C has been reported to have antiviral activity against influenza virus A2/Japan-305 [27].

Other activities
In vitro experiments have demonstrated inhibition of hyaluronidase activity by hederagenin (but not by hederacoside C or α-hederin) [28], anthelminthic activity of ivy leaf extracts [29], antileishmanial activity of ivy leaf saponins [30-32] and anti-trypanosomial activity of α-hederin and hederagenin (but not hederasaponin C) [33]

In vivo experiments

Spasmolytic activity
In the compressed air model in conscious guinea pigs, an orally administered ethanolic extract from ivy leaf at 50 mg/kg body weight dose-dependently inhibited bronchoconstriction induced by inhalation of ovalbumin (57% inhibition, $p = 0.01$) or platelet

activating factor (43% inhibition, p = 0.03) [34].

Anti-inflammatory activity
An orally administered ethanolic extract from ivy leaf at 162 mg/kg body weight inhibited carrageenan-induced rat paw oedema by 39% after 1 hour and by 5% after 5 hours [34]. A saponin mixture isolated from ivy leaf, administered intravenously, inhibited ovalbumin-induced rat paw oedema with an ED_{50} of 0.32 mg/kg [35].

Antifungal activity
Candida albicans infections, as abscesses on the backs of mice, were eliminated after oral administration of a saponin mixture (60% hederasaponin C) from ivy leaf at 50 mg/kg body weight for 10 days. At the same dose level and duration, α-hederin eliminated the infection in 90% of animals and hederasaponin C in 40% of animals. In comparison, the infections were eliminated by oral amphotericin B at 2.5 mg/kg within 6 days [25].

Other effects
Anthelmintic properties of ivy leaf [29] and hepatoprotective properties of α-hederin [36,37] have been demonstrated in mice.

Clinical studies
In early clinical studies, ivy leaf extracts were used in the treatment of children and adults suffering from various respiratory complaints involving coughing. Reductions were observed in the frequency of coughs [38-44].

In a randomized, double-blind, comparative study, 99 patients (aged from 25 to 70 years) with mild to moderate, simple or obstructive, chronic bronchitis were treated daily for 4 weeks with either an oral liquid containing ivy leaf dry extract (5-7.5:1, ethanol 30% m/m; 2 g of dry extract per 100 ml) [3-5 × 20 drops of the oral liquid and 3 × 1 placebo tablet] or ambroxol [3-5 × 20 drops placebo and 3 × 1 ambroxol 30 mg tablet]. Improvements in spirometric and auscultation parameters were observed in both groups with no significant differences between groups. The patients' diaries indicated a tendency towards greater decreases in frequency of coughing, sputum production and dyspnoea in the ivy leaf extract group [5].

In a randomized, comparative, crossover study, 26 children (aged 5 to 11 years) suffering from bronchial asthma were treated for 3 days with preparations containing a 30%-ethanolic dry extract (5-7.5:1) from ivy leaf: 2 × 25 drops of an oral liquid preparation daily (= 35 mg of the extract daily) and then, after a wash-out interval, 2 suppositories daily (= 160 mg of the extract daily), or vice versa. Compared to initial values, reductions of 31% (oral liquid) and 23% (suppositories) in obstruction of the airways was observed. Both dosage forms were well tolerated [8].

In a randomized, double-blind, placebo-controlled, crossover study, 24 patients aged 4-12 years suffering from bronchial asthma were treated with an oral liquid containing ivy leaf dry extract (2 × 25 drops, corresponding to 35 mg of the extract or 210 mg of crude drug daily) for 3 days and, over a separate 3-day period, with placebo drops. A significant reduction in airway resistance was observed in the verum group (p = 0.0361) in comparison with the placebo group [9].

In an open multicentre study involving 52 children (aged up to 12 years) suffering from bronchial complaints, the above-mentioned oral liquid preparation containing ivy leaf dry extract (5-7.5:1; ethanol 30% m/m) was compared with another containing ivy leaf dry extract (3-6:1; ethanol 60%; 200 mg of hederacoside C per 100 ml). The daily dose was: children up to 4 years, 2 × 5 ml daily; 4-10 years, 2 × 7.5 ml daily; 10-12 years, 2 × 10 ml daily. Treatment for 10 days led to improvement of symptoms in both groups with no significance differences between groups [14].

In a open pilot study, 26 children (aged 4-10 years) with chronic, obstructive bronchitis were treated with an ethanol-free oral preparation of ivy leaf extract (4 × 1-2 teaspoonsful daily) for 4 weeks. Spirometry results, auscultatory findings and symptoms such as cough, sputum and dyspnoea improved after the first week in most of the children. Good to very good efficacy was reported in more than two-thirds of the children and no adverse reactions were reported [4].

In a multicentre surveillance study, 113 children (aged 6-15 years) suffering from recurrent obstructive respiratory complaints were treated with an ethanol-free oral preparation of ivy leaf extract for up to 20 days (in some cases up to 30 days). As the daily dose the majority took 6 × 2.5 ml, one-third took 8-10 × 2.5 ml and a few took only 3-4 × 2.5 ml. Compared to baseline, improvements were observed in lung function and accompanying symptoms of coughing and expectoration. The physicians concluded that the optimal daily dosage was 6 × 2.5 ml [6].

In a randomized, double-blind, crossover study involving 25 children (aged 10-15 years) with chronic obstructive pulmonary complaints, changes in lung function were examined after treatment over separate 10-day periods with two oral liquid preparations based on the same ivy leaf dry extract: an ethanol-free preparation (3 × 5 ml daily, corresponding to 3 × 35 mg of dry extract or 630 mg of crude drug daily) and an ethanol-containing preparation (3 × 20 drops daily, corresponding to 3 × 14 mg of dry extract or 252 mg of crude drug daily). Comparable improvements in spirometric and body-plethysmographic parameters were observed after both treatments. However, higher dosages of the ethanol-free preparation were required

to achieve a therapeutic effect equivalent to that of the ethanol-containing preparation [7].

In an open comparative study, children aged 10-14 years suffering from chronic obstructive bronchitis were treated daily for 3 days with two different oral liquid preparations containing ivy leaf dry extract, both dosages corresponding to 250 mg of dried ivy leaf per day: an ethanolic preparation and an ethanol-free preparation. The spirometry results showed that, despite identical dosages in terms of crude drug, improvements in lung function after taking the ethanolic preparation were clearly superior to those after the ethanol-free preparation, with increases in 1-second capacity (FEV_1) of 18% and 8.2% respectively ($p<0.05$) [13].

In an open study 372 children (aged from 2 months to over 10 years, average 5.7 years) suffering from respiratory tract infections were treated for 7 days with an ethanol-free oral liquid preparation containing a dry extract from ivy leaf (6-7:1, ethanol 40%; 2 ml of preparation contained 18 mg of extract corresponding to 108-126 mg of crude drug). Depending on age, average daily doses ranged from 2.8 ml to 6.7 ml. Compared to baseline, substantial improvements were observed in lung function and cough symptoms, and the physicians rated efficacy as good to very good in 94.4% of patients [10].

In an open study, 1024 children suffering from acute infections of the upper respiratory tract (52.4%), acute bronchitis/bronchiolitis (26.6%) or bronchitis (not further specified, 22.2%) were treated with an ivy leaf dry extract. Compared to initial values, significant reductions were observed in coughing, expectoration and airway resistance ($p<0.01$) [11].

Pharmacokinetic properties

No data available.

Preclinical safety data

Single dose toxicity

The oral LD_{50} of several ivy leaf extracts in mice was determined as > 3 g/kg body weight [45]. Oral administration of a dry extract of ivy leaf (ethanol 66% V/V) to rats at up to 4.1 g/kg body weight caused no deaths within 72 hours; only diarrhoea was observed [3,46].

Oral LD_{50} values in mice of saponin mixtures from ivy leaf containing 60% and 90% of hederacoside C, and of hederasaponin C and α-hederin, were all > 4 g/kg body weight; the intraperitoneal LD_{50} values for α-hederin and the saponin mixture containing 60% of hederacoside C were 1.8 and 2.3 g/kg respectively [25]. In an earlier study, oral and intravenous LD_{50} values in rats for crude saponins from ivy leaf were reported as > 100 mg/kg and 13 mg/kg respectively [35].

Repeated dose toxicity

Daily oral administration of an ivy leaf dry extract to rats at 1.5 g/kg body weight for 100 days caused no toxic effects; haematological and biochemical parameters, histological findings and kidney and liver weights were normal compared to those of control animals [47]. Haemolytic effects were detected after oral administration of a hydroethanolic dry extract from ivy leaf to rats at 4 g/kg body weight for 90 days [45].

Mutagenicity and cytotoxicity

α-hederin, β-hederin and δ-hederin isolated from ivy leaf showed no mutagenic potential in the Ames test using *Salmonella typhimurium* strain TA 98, with or without S9 activation. These three saponins showed dose-dependent antimutagenic effects against benz[*a*]pyrene at levels between 80 and 200 µg/plate in the Ames test [48]. In another study, α-hederin prevented gene mutations caused by doxorubicin in human lymphocytes [49].

Cytotoxic properties of α and β-hederin have been demonstrated in mouse 3T3 non-cancer fibroblasts, mouse B16 melanoma cells and human HeLa tumour cells [50,51]. In the presence of serum albumin, the cytotoxic effect decreased. α-Hederin also induced vacuolisation of the cytoplasma and membrane alterations leading to cell death [51].

Reproductive toxicity

No data available on ivy leaf or extracts from it.

Intoxication of the maternal animal causes an acute phase reaction characterized by redistribution of the trace elements zinc, copper and iron, and by an increase in various plasma and liver proteins (e.g. metallothionein, α_1-acid glycoprotein and ceruloplasmin), associated with non-specific malformations in the embryo. Subcutaneous administration of α-hederin at 3 to 300 µmol/kg body weight to pregnant rats on gestation days 8 and/or 11 induced an acute phase response indicated by decreased concentrations of Fe and Zn and increased concentrations of Cu, α_1-acid glycoprotein and ceruloplasmin in plasma along with a dose-dependent increase in the concentration of maternal hepatic metallothionein (MT). The maximum induction of MT was 11- to 15-fold greater than in controls after doses of 30 µmol/kg or higher. Doses of both 30 and 300 µmol/kg significantly increased resorption incidence ($p = 0.05$), and 300 µmol/kg body weight also decreased fetal weight and increased the incidence of abnormal fetuses [52].

In another study α-hederin was subcutaneously administered to rats at 20 and 30 µmol/kg body

weight daily on gestation days 6-15, resulting in sustained elevation of hepatic metallothionein and subsequent redistribution of zinc. This led to a decrease in the zinc available to the embryo and ultimately to adverse development of the offspring. Repeated dosing throughout organogenesis increased the severity of the effects previously observed with single large doses of α-hederin administered during mid-gestation [53].

Addition of α-hederin to an embryo culture, directly (300 µmol) or as serum collected 2 hours after administration of α-hederin to the maternal rat (i.e. before the onset of MT synthesis), had no embryotoxic effect. However, after addition of serum obtained at the peak of metallothionein synthesis (18 hours after application of α-hederin to the maternal animals), the embryos developed normally only after addition of zinc [52].

The above studies showed that single high toxic doses, or repeated low doses, of α-hederin alter (as do many other substances) systemic zinc distribution in the pregnant rat, which is associated with abnormal embryo development. Abnormalities of embryogenesis due to the amount of α-hederin orally administered in therapeutic doses of ivy leaf extracts (with absorption to a lesser extent) cannot be deduced from these results.

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HIPPOCASTANI SEMEN

Horse-chestnut Seed

DEFINITION

Horse-chestnut seed consists of the dried seeds of *Aesculus hippocastanum* L. containing not less than 3.0 per cent of triterpene glycosides, expressed as anhydrous aescin ($C_{55}H_{86}O_{24}$; M_r 1131) and calculated with reference to the dried drug.

The material complies with the monographs of the French [1] and German [2] pharmacopoeias.

Draft monographs on Horse-chestnut [3] and Standardised Horse-chestnut Dry Extract [4], intended for the European Pharmacopoeia, have been published.

CONSTITUENTS

The characteristic constituents, collectively known as aescin (3-10%), are a mixture of acylated triterpene glycosides (saponins) based on two main aglycones, protoaescigenin and barringtogenol C, which differ only in that protoaescigenin has a hydroxyl group on C-24. All the saponins have a trisaccharide group at C-3, comprising glucuronic acid with substituent sugars (glucose, galactose or xylose) at the 2- and 4-positions. The two major saponins, both arising from protoaescigenin, are esterified at the 21β-position, one with angelic acid, the other with tiglic acid, and with acetic acid at the 22α-position [5-11].

More than 30 different saponins have been identified in aescin. They can be fractionated into: β-aescin, containing only 22-*O*-acetyl compounds; cryptoaescin, containing only 28-*O*-acetyl compounds; and α-aescin, which is a mixture of β-aescin and cryptoaescin [6,8,11,12].

Other constituents include flavonoids, principally di- and triglycosides of quercetin and kaempferol (0.3%) [13], sterols [14,15], essential oil [16] and a high proportion of starch (30-60%) [11].

The seed pericarp contains saponins based on two aglycones, barringtogenol C and R_1-barrigenol [17], and proanthocyanidins [18-20].

CLINICAL PARTICULARS

Therapeutic indications

Chronic venous insufficiency, varicosis [11,21-33].

E/S/C/O/P
MONOGRAPHS
Second Edition

Posology and method of administration

Dosage

Adult daily dose: drug or hydroalcoholic extract containing 50-150 mg of triterpene glycosides (calculated as aescin), usually in divided doses [21-33].
Elderly: dose as for adults.

Not recommended for children.

Method of administration
For oral administration in solid or liquid preparations.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use.
None required.

Interactions with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Horse-chestnut seed extracts have been used in clinical studies involving pregnant women [21,25,27,28], with some studies excluding those in the third trimester [25,28]. No adverse effects have been reported but, in accordance with general medical practice, the drug should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In rare cases gastric irritation or pruritus may occur [11,27,33,34].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Note: HCSE is used as an abbreviation for "horse-chestnut seed extract" in the following text.

Pharmacodynamic properties

Anti-inflammatory and anti-oedematous effects

In vitro experiments
A saponin fraction from horse-chestnut seed inhibited the activity of prostaglandin synthetase [35].

In vivo experiments
Aescin significantly inhibited egg albumin-induced rat paw oedema ($p<0.001$) when administered intravenously at 0.2 and 2.5 mg/kg body weight [36].

Aescin administered intraperitoneally at 4 mg/kg body weight completely inhibited dextran-induced exudative oedema on rat paw skin; the effect was considered to be due primarily to the potent vaso-constrictive activity of aescin [37].

In carrageenan-induced rat paw oedema a saponin fraction from horse-chestnut seed, administered intravenously at 3.75 mg/kg body weight, inhibited inflammation; it also showed significant analgesic activity when administered orally at 7.5 mg/kg or intravenously at 3.75 mg/kg ($p<0.001$) [35].

When aescin was injected at 2.5 mg/kg into the tail veins of rats, which were subsequently treated intraperitoneally with 50% egg white solution (24 ml/kg) and 1% Evans Blue (5 ml/kg, intravenously), the dye remained mainly in the mesenteric vessels, whereas in control animals a high concentration of dye was found in the abdominal cavity. Thus aescin effectively prevented an increase in vascular permeability caused by egg white injection [36].

Pharmacological studies in humans
The effect of HCSE on transcapillary filtration has been assessed by measuring capillary filtration coefficients in two randomized, placebo-controlled studies [38,39]. In the first study, oral administration of a single dose of HCSE (300 mg; n = 12) or placebo (n = 14) to healthy volunteers produced a significantly lower capillary filtration coefficient in the verum group [38]. In the second study, which had a double-blind, crossover design and involved 22 patients with chronic venous insufficiency, 3 hours after oral administration of a single dose of HCSE (600 mg, standardized to 100 mg of aescin) the capillary filtration coefficient had decreased significantly by 22% ($p = 0.006$), compared to a slight increase with placebo [39]. Both studies demonstrated the oedema-protective effect of HCSE, induced by an increase in capillary resistance.

Effects on venous tone

In vitro experiments
HCSE (standardized to 16% aescin) and pure aescin in concentrations of 5-150 µg/ml had no effect on the tone of isolated veins from cows (vena metacarpalis) or humans (vena saphena). However, higher concentrations of the extract (0.2 mg/ml) and aescin (0.1 mg/ml) slowly induced a contraction, which was irreversible even 3 hours after exposure. The effect was comparable to that of a standard saponin (Merck) tested in the same experiment [40].

Similar effects on venous tone were observed with

HCSE at 0.2-1 mg/ml on isolated veins of rabbits [41] and with aescin at 1 ng/ml to 1 mg/ml on human vein (vena saphena) preparations [42]. The effects were comparable to those of essential phospholipids [41], serotonin or dihydroergotamine [43], and significantly greater than those of acetylcholine or vasopressin [42].

The above results could be reproduced on isolated human vein (vena saphena) and rabbit portal vein with aescin at 5-10 mg/ml; these lower concentrations increased the tonus of the veins [44]. The effect is partly explained by the ability of aescin to enhance $PGF_{2\alpha}$ generation in the veins. It has been suggested that the prostaglandins act primarily as local hormones in the regulation of vascular reactivity [44].

In the isolated canine saphenous vein, HCSE induced concentration-dependent contractions at concentrations above 5×10^{-5} mg/ml; the contraction at 5×10^{-4} mg/ml reached a maximum in 15 minutes and lasted more than 5 hours [45].

In vivo experiments
HCSE administered intravenously at 50 mg significantly increased femoral venous pressure ($p<0.001$) in anaesthetized dogs [45].

In the canine saphenous vein perfused in the opposite direction to blood flow, HCSE decreased the flow in a dose-dependent manner, 100 mg producing an effect comparable to that of 10 µg of noradrenaline, demonstrating that it can ensure closure of valves [45].

HCSE orally administered to rats at 200 mg/kg significantly decreased cutaneous capillary hyperpermeability induced by histamine or serotonin ($p<0.001$) [45].

Pharmacological studies in humans
In a study of venous tone, a single dose of 150 mg of HCSE was administered orally to 23 healthy young subjects. A further 14 subjects received either 80 mg of HCSE or identical placebo capsules in a double-blind, crossover design. Plethysmographic measurements taken before and 2 hours after administration showed that both dosages of HCSE significantly increased venous tone and decreased venous capacity [46].

Comparable results were obtained from a further study in which 12 healthy volunteers firstly received placebos and then a single oral dose of HCSE (360 mg, standardized to 90 mg of aescin). In contrast, intravenous administration of 20 mg of aescin had no effect on venous tone [47].

Effects on lysosymal enzymes

In vitro experiments
Aescin showed inhibitory activity (IC_{50}: 149.9 µM) on hyaluronidase, which promotes the degradation of hyaluronic acid, the main component of the extravascular matrix surrounding capillary walls; its activity was considerably greater than that of its genin, aescinol (IC_{50}: 1.65 mM) [48].

Pharmacological studies in humans
Three hydrolases, β-N-acetylglucosaminidase, β-glucuronidase and arylsulphatase, catalyze the breakdown of proteoglycans, which constitute part of capillary walls. In the serum of varicose in-patients the activity of these enzymes has been found to be markedly increased (by 60-120%) compared to healthy subjects; this may render the capillaries more permeable and fragile [49]. In two studies, one with 10 patients [49] and the other with 15 patients [50], oral administration of HCSE (900 mg, standardized to 150 mg of aescin) daily for 12 consecutive days led to significant reductions in the activity of these enzymes ($p<0.01$ and $p<0.05$ respectively), of the same order of magnitude (about 30%) for each enzyme. It was hypothesized that HCSE does not inhibit the individual enzymes but has a protective action towards the site of enzyme release, the fragile lysosomal membrane. The observed enzyme inhibition should reduce degradation of the proteoglycans, providing an explanation for the effect of HCSE on capillary permeability and capillary resistance [51].

Effects on blood flow

Pharmacological studies in humans
Treatment of 30 varicosis patients with oral HCSE (1800 mg daily) for 12 days led to a 30% increase in flow velocity of venous blood between the instep and groin, as quantitatively determined by the "^{133}Xe appearance method". Blood viscosity was reduced at the same time. The favourable effects on haemodynamics correlated with improvements in subjective complaints in 73% of the cases [43].

Effects on haematoma

Pharmacological studies in humans
The efficacy of a topically applied gel containing 2% of aescin in reducing the tenderness to pressure of haematoma (experimentally induced by injection) was demonstrated in a randomized, double blind, placebo-controlled, single dose study involving 70 healthy subjects (34 verum, 36 placebo). Based on tonometric sensitivity measurements, the aescin gel significantly reduced tenderness to pressure ($p<0.001$) and this effect was observed from 1 hour after treatment until the end of the 9-hour study period [52].

Clinical studies

Anti-inflammatory and anti-oedematous effects
The anti-oedematous effect of various HCSE preparations, standardized on aescin and administered

orally, has been proven in a number of clinical studies on patients suffering from chronic venous insufficiency or varicosis [21-33].

In three double-blind, placebo-controlled, crossover studies [21-23], twice daily oral administration of a preparation containing HCSE (300 mg, standardized to 50 mg of aescin) for 20-21 days produced significant improvement in symptoms compared to placebo.

In the first crossover study, based on objective and subjective assessments on 96 female patients with varicosis of varying etiology, significant improvements ($p<0.001$) were noted over a range of symptoms such as oedema, inflammation, pruritus, tenderness and pigmentation [21].

In the second crossover study, subjective assessment of 226 predominantly female patients with varicosis showed significant improvements in symptoms of oedema, pain and pruritus ($p<0.01$ or 0.05) [22].

In the third crossover study, on 95 patients with varicosis or chronic venous insufficiency, subjective assessments revealed significant improvements in oedema, systremma, pain and fatigue or heaviness of the legs ($p<0.01$ or $p<0.05$), but not in pruritus [23].

Measurements of leg or foot volume (with a hydroplethysmometer) and/or circumferences, in some cases accompanied by assessment of subjective symptoms, were used to demonstrate the anti-oedematous and oedema-protective effects of oral treatment with HCSE in the following studies [24-33].

39 patients with venous oedema attributed to chronic venous insufficiency were treated with either HCSE (standardized to 150 mg of aescin; n = 20) or placebo (n = 19) daily for 6 weeks in a randomized, double-blind study. Leg volume before and after oedema provocation, i.e. subjecting the leg to stress in the form of a haemostatic load, was measured at 2-week intervals. A significant reduction in average leg volume from 1565 ml to 1481 ml was observed in the verum group ($p<0.01$) over the 6-week period. The results were more discernible after oedema provocation, demonstrating that the extract has a venoprotective effect under practical conditions of daily activity. These observations were corroborated by auxiliary tests and subjective assessments [24].

In a randomized, double-blind, crossover study, 20 female patients between 20 and 40 years of age with varicosis during pregnancy (excluding patients in their third trimester) or attributed to chronic venous insufficiency stage I received either HCSE (600 mg extract, standardized to 100 mg of aescin) or placebo daily for 2 weeks. A reduction in leg volume after verum treatment was significant ($p = 0.009$); improvements in the patients who received verum treatment first were eliminated in the subsequent placebo phase. Leg circumferences and subjective symptoms were also significantly reduced during verum therapy ($p<0.05$). In assessment of efficacy the verum treatment was rated as significantly better than placebo by both the physicians ($p<0.01$) and the patients ($p<0.05$) [25].

30 outpatients with symptoms of chronic venous insufficiency including peripheral oedema received either HCSE (600 mg, standardized to 100 mg of aescin; n = 15) or placebo (n =15) daily for 20 days in a randomized, double-blind study. A significant reduction in leg circumference ($p<0.05$), and hence in oedema, was observed in the verum group [26].

In a randomized, double-blind, crossover study, 50 pregnant women (stage of pregnancy not stated) with oedema due to chronic venous insufficiency received either HCSE (480-580 mg, standardized to 100 mg of aescin) or placebo daily for 20 days. Significant reductions in foot volume ($p<0.01$) before and after oedema provocation were observed after verum treatment; the circumferences at heel, ankle and calf paralleled these changes. Subjective symptoms (pain, fatigue, swelling, itching) were also less severe after verum treatment [27].

In a randomized, double-blind, crossover study, 20 patients with pregnancy-related varicosis (excluding patients in their third trimester) or chronic venous insufficiency received, in two 14-day treatment phases separated by a 5-day wash-out period, HCSE (480-580 mg daily, standardized to 100 mg of aescin) and then placebo, or vice versa. Significant reductions in leg volume ($p = 0.0009$) were observed after verum treatment. Decreases in leg circumference and improvements in subjectively assessed symptoms correlated with this finding [28].

In a randomized, double-blind comparative study 40 patients suffering from chronic venous insufficiency and peripheral venous oedema received, after a one-week placebo run-in period, either HSCE (standardized to 150 mg of aescin; n = 20) or a preparation providing 2000 mg of *O*-(β-hydroxyethyl)-rutoside (n = 20) daily for 8 weeks. Based on leg circumferences (measured both before and after an oedema-provoking procedure with legs hanging free), the two preparations had a similar and favourable effect in reducing oedema over 8 weeks of treatment, but the HSCE preparation had a more pronounced effect [29].

Patients with chronic venous insufficiency received either HCSE (standardized to 100 mg of aescin; n = 19) or placebo (n = 20) daily for 4 weeks in a double-blind study. Foot and lower leg volume was significantly reduced in the verum group ($p<0.01$ after 14 days, $p<0.001$ after 28 days); this was the case both in normal blood flow and in pronounced ischaemia. Subjective symptoms (pain, tiredness, feeling of tension

and pruritus in the legs) and global assessment of the treatment by physicians and patients also showed significant improvement in the verum group. There were no differences between verum and placebo with regard to venous capacity or venous drainage when the leg was elevated [30].

In a randomized study, 240 patients with substantial lower leg oedema due to chronic venous insufficiency received, after a 2-week placebo run-in period, one of three treatments daily for 12 weeks: HCSE providing 100 mg of aescin (n = 95), compression therapy with elastic compression stockings (n = 99) or placebo (n = 46). The study was blinded in the HCSE and placebo groups. Patients allocated to compression treatment received a diuretic daily during the first 7 days and thereafter were provided with individually fitted class II compression stockings. Based on measurement of lower leg volumes in the more severely affected leg, significant oedema reductions were achieved in the HCSE ($p = 0.005$) and compression therapy ($p = 0.002$) groups compared to the placebo group, and the two verum therapies were shown to be equivalent ($p<0.001$). It was concluded that HCSE offers an alternative to compression for the treatment of oedema resulting from chronic venous insufficiency; a 12-week course of HCSE may provide a 25% reduction in mean oedema volume [31].

In a randomized, double-blind crossover study, 50 patients with varicosis and oedema attributed to chronic venous insufficiency received, after a one-week placebo run-in phase, an unspecified HCSE preparation (one sustained release tablet twice daily) for 2 weeks and then, after a one-week wash-out phase, placebo, or vice versa. A significant decrease in leg volume ($p<0.01$) was achieved after verum treatment compared to placebo [32].

In a randomized, double-blind study, 137 female postmenopausal patients with chronic venous insufficiency were given one of the following treatments (plus placebo tablets and/or capsules of identical appearance to the alternative treatments):

a) 600 mg of HCSE standardized to 100 mg of aescin, daily for 12 weeks (n = 51),
or
b) a standardized mixture of oxerutins, 1000 mg daily for 12 weeks (OX1000; n = 51),
or
c) a standardized mixture of oxerutins, 1000 mg (loading dose) daily for 4 weeks, followed by 500 mg (maintenance dose) daily for 8 weeks (OX1000-500; n = 35).

After a one-week placebo run-in and 12 weeks of treatment, there was a follow-up period of 6 weeks without treatment. Reductions in mean leg volumes after 18 weeks, expressed as area under baseline (ml.d), were 3004 for HCSE, 5273 for OX1000 and 3187 for OX1000-500. The numerical superiority of oxerutins seemed to be based not on a higher intrinsic potency but on a higher responder fraction of the study population. It was concluded that 600 mg of HCSE or 1000 mg of oxerutins daily are effective in treatment of chronic venous insufficiency and able to produce a mean reduction in leg volume of about 100 ml after 12 weeks in responding patients. This represents a therapeutically relevant reduction in oedema, comparable to values reported for compression therapy [33].

The conclusions from a criteria-based systematic review of 13 randomized, double-blind, controlled clinical studies (8 placebo-controlled and 5 controlled against reference medications) were that the evidence implied that:

- HCSE is superior to placebo and as effective as reference medications in alleviating the objective signs and subjective symptoms of chronic venous insufficiency.
- HCSE is safe and effective as a symptomatic, short-term treatment of chronic venous insufficiency.

Publication bias and methodological shortcomings were considered important caveats to these conclusions, with more rigorous randomized controlled trials required to verify the usefulness of the treatment, especially for long term use and as an adjunct to compression therapy [53].

Pharmacokinetic properties

Pharmacokinetics in animals

After intravenous administration of ^{3}H-aescin to rats and mice the concentrations in blood declined rapidly during the first few hours and more slowly thereafter. Elimination took place via the liver and kidneys. One hour after injection about 30% of the dose had been excreted, two thirds in bile and one third in urine, mostly as the parent drug. Organ distribution studies showed higher concentrations than in the blood only in the excretion organs, while the CNS was almost free from aescin [54,55].

After oral administration to rats and mice ^{3}H-aescin was absorbed to the extent of 10-15% of the dose, measured by the amounts of aescin and metabolites excreted in bile and urine. Aescin seemed to be absorbed mainly from the duodenum. The percentage of metabolites was higher than after intravenous injection; their chromatographic profile appeared partly aescinol-like and partly as aglycones [54,55].

Pharmacokinetics in humans

After intravenous administration the pharmacokinetics of aescin correspond to an open three compartment model. With an intravenous dose of 5 mg of aescin (infusion rate: 718 µg/min) the elimination half-life $t_{0.5}\alpha$ was 6.6 minutes; $t_{0.5}\beta$ was 1.74 hours and $t_{0.5}\gamma$ was 14.36 hours. The distribution volume under

steady state conditions was 100.9 litres, total plasma clearance 21.8 ml/min and renal clearance 1.7 ml/min. Urinary excretion from 0 to 120 hours after injection comprised 8.2% of the dose [34].

After oral administration of an aescin solution the absolute bioavailability was determined as only 1.5%. This low bioavailability is due to a pronounced first pass effect (metabolism and biliary excretion). The relative bioavailability of aescin from a horse-chestnut seed extract was 100% compared to an aescin solution [34].

No significant differences in relative bioavailability between rapid and slow release oral dosage forms of HCSE were seen by Kunz et al. [56,57] whereas Schrader et al. [58] and Dittgen et al. [59] noted higher bioavailability with the rapid release form. Maximum plasma concentrations were found between 1.8 and 3.3 hours [57-61] irrespective of the type of preparation. Terminal plasma half-lives were calculated as 17.8-21.2 hours [60] and 18.5-24.0 hours [59] respectively. Only 0.1% of the dose could be detected in urine [34]. The pharmacokinetics and bioavailability of aescin in various extract preparations have been reviewed by Loew et al. [62,63].

In a recent randomized, open, crossover study, 18 healthy volunteers received an oral rapid-release tablet formulation (2 × 300 mg of HCSE, providing 2 × 50 mg of aescin, daily for 7 days) and subsequently, without a wash-out period, a prolonged-release capsule formulation as a reference preparation (2 × 240-290 mg of HCSE, providing 2 × 50 mg of aescin, daily for 7 days), or vice versa. Blood samples covering a full 24-hour cycle were taken on the 7th day in each test period for steady-state pharmacokinetic profiling using a specific radioimmunoassay for serum concentrations of β-aescin. With both formulations, C_{max} after the first dose of the day was in the range 16.7-18.5 ng/ml, t_{max} was 2.1 hours and $C_{average}$ 9.9-10.6 ng/ml. Somewhat lower values for C_{max} (10.2-11.7 ng/ml) and $C_{average}$ (7.0-7.1 ng/ml) after the second dose of the day were attributed to the effects of food. The two formulations proved bioequivalent with respect to the extent of absorption of aescin; AUC values (0-24 hours) ranged from 84% to 114% (90% confidence interval; point estimate 98%) [57].

Plasma protein binding of aescin was determined to be 84% [34]; binding to erythrocytes can be ignored [64]. In agreement with the animal studies [54] a blood-brain barrier for aescin was also apparent in humans [64].

Preclinical safety data

Acute toxicity

LD_{50} values for HCSE determined in various laboratory animals are given in Table 1. Dogs were also tested but the oral LD_{50} could not be established since doses of more than 130 mg/kg body weight caused vomiting of the substance shortly after administration [65].

TABLE 1
LD_{50} values of horse-chestnut seed extract in mg/kg body weight [65]

Animal	Mode of Administration		
	Oral	Intra-peritoneal	Intra-venous
Mouse	990	342	138
Rat	2150		165
Guinea pig	1120		465
Rabbit	1530		180

The oral LD_{50} of an aqueous extract (1:1) from horse-chestnut seed was determined as 10.7 and 10.6 g/kg body weight in adult Syrian hamsters and two-week-old chicks respectively [66].

Sub-acute intravenous toxicity

Daily intravenous doses of HCSE at 9, 30 or 90 mg/kg body weight were administered to rats for 8 weeks. With the 90 mg/kg dose, 8 out of 30 animals died during the first few days but the others developed normal body weights; 9 mg/kg was tolerated virtually without symptoms [65]. The no-effect intravenous dose level was considered to be around 30 mg/kg body weight, approximately 7 times the daily therapeutic dose given orally to humans [34].

Chronic oral toxicity

Neither toxic effects nor organ damage were observed after 34-week oral administration of HCSE to dogs (2 male, 2 female per dose and control group) at 20, 40 or 80 mg/kg body weight daily (5 days per week) and to rats (20 male, 20 female per dose and control group) at 100, 200 and 400 mg/kg body weight daily [65].

The highest dose level used in dogs corresponds to 8 times, and that used in rats to 40 times, the usual daily therapeutic dose in humans.

Teratogenicity

No significant effects compared to control animals were observed in teratogenicity studies involving daily oral administration of HCSE at 100 mg/kg body weight to rats and rabbits or at 300 mg/kg to rats. At

300 mg/kg in rabbits a significant reduction ($p<0.001$) was apparent in the mean body weight of foetuses, 25.2 g compared to 31.2 g in control animals [65]. However, 300 mg/kg body weight is approximately 30 times the recommended therapeutic dose in humans [34].

Mutagenicity

In the Ames mutagenicity test, using *Salmonella typhimurium* strain TA98, HCSE gave a negative response without activation, but a weakly positive response (factor 2-3) with S9 activation. Fluid extracts of horse-chestnut seed gave a weakly positive response (factor 2-3) without activation and a negative response with activation. The authors suggested that quercetin is possibly the main mutagenic principle in these extracts [67]. However, the potential genotoxicity of quercetin, an ubiquitous substance found in many fruits and vegetables (daily intake from food estimated as at least 25 mg), has been extensively studied and the results have been interpreted as not relevant to humans [68].

Clinical safety data

From controlled studies in patients with chronic venous insufficiency the rate of adverse reactions from HCSE has ranged from 0.9 to 3.0%, mainly involving gastrointestinal complaints, dizziness, headache or itching. In a large observational study, only 0.6% of patients reported adverse events, consisting mainly of gastrointestinal disturbances. Horse-chestnut seed preparations and aescin are therefore considered to have excellent tolerability [69].

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HYPERICI HERBA

St. John's Wort

DEFINITION

St. John's wort consists of the whole or cut, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time. It contains not less than 0.08 per cent of total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

The characteristic constituents are naphthodianthrones and phloroglucinols. Naphthodianthrones (0.05-0.3%), consisting mainly of hypericin and pseudohypericin, accumulate primarily in the flowers and buds [2,3]; lower levels than 0.1% may result from harvesting of lower parts of the herb. Other naphthodianthrones present, and included in the term 'total hypericins', are the biosynthetic precursors protohypericin and protopseudohypericin (which are transformed into hypericin and pseudohypericin respectively on exposure to light), and a small amount of cyclopseudohypericin [4-6].

The principal phloroglucinols are hyperforin (2-4.5%) and adhyperforin (0.2-1.8%) [7-9]. Both compounds have limited stability [10-14] and their oxidated derivatives are also present [9].

Quercetin glycosides (2-4%), including hyperoside, quercitrin, isoquercitrin and rutin, are the main flavonoids [2,15-17]; a small amount of free quercetin is also found [17]. Biflavonoids such as I3,II8-biapigenin (0.1-0.5%) and I3',II8-biapigenin (amentoflavone, 0.01-0.05%) occur exclusively in the flowers [2,17-19].

Other constituents include phenylpropanoids, such as chlorogenic acid and other caffeoylquinic and *p*-coumaroylquinic esters [2,20]; condensed tannins and oligomeric proanthocyanidins [2,3] including procyanidins A2, B1, B2, B3, B5, B7 and C1 [21,22], together with catechin and epicatechin monomers [2,21]; trace amounts of xanthones such as 1,3,6,7-tetrahydroxyxanthone (0.0004% in the leaves and stems) [2,23]; and essential oil (0.1-0.25%), containing mainly higher n-alkanes and monoterpenes [2].

E/S/C/O/P
MONOGRAPHS
Second Edition

CLINICAL PARTICULARS

Therapeutic indications

Preparations based on hydroalcoholic extracts (50-60% ethanol or 80% methanol) and tinctures (49-50% ethanol)
Episodes of mild depressive disorders [24-31] or mild to moderate depressive episodes in accordance with ICD-10 categories F32.0, F32.1, F33.0 and F33.1 (see the boxes below) [32-45].

Other preparations
Mild depression [46]; support of emotional balance [47].

Posology and method of administration

Dosage

Preparations based on hydroalcoholic extracts (50-60% ethanol or 80% methanol)
Adults and children from 12 years: 450-1050 mg daily of hydroalcoholic dry extracts with drug-to-extract ratios of 2.5-5:1, 4-7:1 or 5-7:1 [28-31,34-41, 43-45, 50,51].

Herbal tinctures and teas
3-4.5 ml daily of tincture (1:5, ethanol 60 % V/V) [24-27,32,33,52,53].
2-4 g of the drug daily for tea infusions [47].

Elderly: dose as for adults

Children from 6 to 12 years under medical supervision only: half the adult dose.

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist for more than 4-6 weeks, seek medical advice.

Contra-indications
Not to be used after organ transplants [54] or by HIV-antibody positive individuals treated with protease-1 inhibitors (e.g. indinavir) [55].

Special warnings and special precautions for use
As with all antidepressant treatments, full manifestation of the therapeutic effect may take 3-4 weeks;

Classification of severity of depression based on ICD-10 criteria [48]

Mild depressive episode: [F32.0/F33.0]	Mild first manifestation or mild recurrence of at least two target signs and at least two associated symptoms
Moderate depressive episode: [F32.1/F33.1]	Two or three target signs and at least three or four associated symptoms (first manifestation or recurrent episode)
Severe depressive episode: [F32.2/F33.2/F32.3/F33.3]	All three target signs and at least four associated symptoms, some particularly pronounced (first manifestation or recurrent, without or with psychotic symptoms)

Diagnostic features of depressive disorders according to ICD-10 Chapter V Primary Care Version [49]

Target signs	*Associated symptoms*
Low or sad mood	Disturbed sleep
Loss of interest or pleasure	Feelings of guilt and unworthiness
Fatigue or loss of energy	Reduced self-esteem and self confidence
	Poor concentration
	Disturbed appetite
	Decreased libido
	Suicidal throughts or acts
	Agitation or slowing of movement or speech
	Weight loss

Symptoms of anxiety or nervousness are also frequently present

there is a risk of suicide, particularly at the beginning of treatment, due to the delay between treatment and clinical improvement. If a significant treatment response in depressive disorders is not apparent after 4 weeks, the medication should be discontinued.

Clinical data on the efficacy of St. John's wort do not support its use in patients with severe major depression or with acute severe depressive disorders [56-58].

It appears advisable to discontinue therapy with St. John's wort in patients to be treated with ciclosporin or indinavir or other antiretroviral substances, and to take particular care in patients on other medication such as antidepressants, anticoagulant therapy (monitoring of clotting time) or therapy with digoxin or theophylline (monitoring of blood levels).

Interaction with other medicaments and other forms of interaction

A number of interactions with preparations of St. John's wort have been reported [7,59,60]. Several types of interaction are involved, such as the so-called serotonin syndrome in cases of concomitant use of St. John's wort preparations with certain antidepressants [61,62] or reductions in blood levels and hence efficacy of other medications. Reduced blood levels have been reported with respect to ciclosporin [54,63,64], indinavir and potentially other antiretroviral protease and transcriptase inhibitors [55], the anticoagulants phenprocoumon and warfarin [65,66], theophylline [67] and digoxin [68].

Induction of several subtypes of the enzyme cytochrome P450 has been discussed as a potential mechanism of the interactions [69-75], but increased expression of the P-glycoprotein drug transporter has also been reported [76].

It remains an open question whether St. John's wort preparations also interact with oral contraceptives, particularly low dose contraceptives (< 50 µg of oestrogen) but a clinical study with a St. John's wort preparation and a contraceptive containing 0.15 mg of desogestrel and 20 µg of ethinylestradiol did not show any sign of an interaction [77].

Pregnancy and lactation

No human data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines

Clinical studies indicate no negative influence on general performance or the ability to drive [78,79].

Undesirable effects

At therapeutic dose levels (up to the equivalent of 6 g of drug), occasional mild gastrointestinal disturbances, nausea, restlessness, fatigue, headache or allergic reactions have been reported [24-47,50-53,56-58,80-93]. Onset of mania was reported in 2 patients with latent bipolar disorders [94]. In one patient taking 500 mg of powdered St. John's wort daily for 4 weeks, stinging pain was experienced on the face, the dorsum of both hands and on arms and legs after exposure to sunlight; the symptoms, which disappeared after discontinuation of the product, were restricted to areas of skin exposed to sunlight; no motor or other sensory disturbances were noted [95]. In another patient, who had ingested 240 mg of St. John's wort extract daily for 3 years, an itchy rash developed on skin areas exposed to light; after discontinuation the symptoms disappeared completely within 2 weeks [96].

In healthy subjects treated with 1800 mg of hydromethanolic extract (containing 5.6 mg of total hypericin) daily for 15 days a slight increase in dermal light sensitivity, evidenced by a change in skin pigmentation, was observed. This could be compensated by reducing light exposure time by 21% [97].

Overdose

Serious phototoxic reactions may occur at much higher dosages than used therapeutically [98]. In healthy subjects, photosensitivity occurred after ingestion of a single dose of 3600 mg (but not after 900 mg or 1800 mg) of a hydromethanolic extract of St. John's wort containing 11.25 mg of total hypericin and experimental exposure to UV-A light, but with only marginal significance ($p<0.03$) compared to placebo [97]. Typical phototoxic symptoms include rash, pruritus and erythema. Treatment consists of avoiding exposure to direct sunlight [99].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamics

In vitro experiments

Effects on enzyme activity

Hydroethanolic extracts of *Hypericum perforatum* have shown inhibition of type A monoamine oxidase (MAO) [100]. Hypericin and St. John's wort preparations have rather low MAO inhibiting activity, whereas xanthones, which are present only in minor quantities (up to 10 ppm) appear to have high MAO-inhibiting activity [101,102].

Inhibition of the enzyme catechol-O-methyl-transferase (COMT) is reported in St. John's wort fractions containing mainly flavonoids [101].

A hydroethanolic extract of St. John's wort, as well as pure hypericin, inhibited the enzyme dopamine-β-

hydroxylase *in vitro* [103]. Dopamine-β-hydroxylase was inhibited by an ethanolic extract from St. John's wort with an IC_{50} of 0.1 µmol/litre (molarity referring to total hypericin content) and by commercially available hypericin with an IC_{50} of 21 µmol/litre; tyrosinase and tyrosine decarboxylase were not influenced by hypericin at 1-10 µmol/litre [104]. In another investigation, dopamine-β-hydroxylase was inhibited by pseudohypericin with an IC_{50} of 3 µmol/litre and by hypericin with an IC_{50} of 5 µmol/litre, whereas the IC_{50} values of various flavonoids were 50 µmol/litre and higher [105].

Receptor binding

Depression has been attributed to reduced availability of serotonin (5-HT), norepinephrine or dopamine in the CNS. Consequently, antidepressants increase the levels of neurotransmitters, e.g. by reuptake inhibition or by inhibiting the degradation. N-Methyl-D-aspartate (NMDA) receptor-mediated processes or binding to opioid receptors have also been considered to be involved in the mechanism of action of conventional antidepressants.

Receptor binding studies with a hydroethanolic extract containing approx. 0.15% of total hypericin revealed moderate interactions with the $GABA_A$/benzodiazepine receptor/chloride-ionophore complex: displacement of ^{3}H-muscimol, ^{3}H-flunitrazepam and ^{35}S-TBPS (t-butyl-bicyclo-phosphorothionate) binding. Pure hypericin resulted in increased binding at the $GABA_A$ and benzodiazepine receptors and at the 5-HT_1 receptor [106].

The biflavonoid amentoflavone has binding activity at the benzodiazepine receptor [107]. A hydromethanolic extract caused reduced expression of serotonin receptors in a neuroblastoma cell line model [108]. Binding to benzodiazepine binding sites in rat brain was inhibited with an IC_{50} of 6.8 µg/ml by a flower extract from St. John's wort, obtained by extraction with methanol followed by acetone, whereas leaf extracts up to 200 µg/ml caused only 25% inhibition of flumazenil binding. The IC_{50} of amentoflavone was 14.9 nM ≅ 7.45 ng/ml, whereas hypericin, flavones and glycosylated flavonoids at concentrations up to 1 µM did not inhibit binding [109].

A St. John's wort extract showed receptor affinity for adenosine (non-specific), $GABA_A$, $GABA_B$, benzodiazepine, inositol triphosphate and MAO-A and -B, whereas pure hypericin had affinity only for NMDA receptors. Only the concentrations required at the $GABA_A$ and $GABA_B$ receptors (between 0.005 and 0.5 µg/ml) were in a relevant dose range. The involvement of GABA in affective disorders is under discussion [110].

The affinity of 1 µM hypericin was determined at 30 receptors or binding sites; more than 40% inhibition was found for non-selective muscarinic cholinergic receptors and for non-selective σ-opioid receptors. Opioid receptor binding of hypericin might be linked to clinical efficacy [111]. Binding of [^{3}H]naloxone to human µ- and rat κ-opioid receptors was inhibited by St. John's wort extracts with IC_{50} values of 25 and 90 µg/ml respectively. Up to a concentration of 10 µM (≅ 5 µg/ml) quercetin, kaempferol and quercitrin were ineffective [112].

Ethanolic extracts produced relatively weak inhibition of binding to µ-, κ- and δ-opioid, $GABA_A$ and oestrogen-α receptors; the opioid binding was inhibited approximately 10 times more potently by methanolic extracts. A hexane fraction strongly inhibited binding to µ-, κ- and δ-opioid receptors and to 5-HT_6 and 5-HT_7 serotonin receptors. Hypericin, pseudohypericin and hyperforin inhibited binding to both opioid and serotonin receptors in the low micromolar range (the naphthodianthrones at 1-4 µM at opioid receptors and 1, 6 or 10 µM at serotonin receptors; hyperforin at 0.4-1 µM at opioid receptors and 2-3 µM at serotonin receptors). Oestrogen binding was inhibited by biapigenin at a low micromolar concentration. The inhibitory effect of the ethanolic extract on $GABA_A$ binding at about 3 µg/ml was not considered to be correlated to antidepressant activity since extracts of *Valeriana officinalis* and *Passiflora incarnata* were similarly active [113].

Reuptake inhibition

A methanolic extract of St. John's wort inhibited serotonin uptake by rat synaptosomes with an IC_{50} of 6.2 µg/ml [114]. A methanolic extract, a CO_2 extract and pure hyperforin were compared for reuptake inhibition in synaptosomal preparations. Pronounced reuptake inhibition for serotonin, noradrenaline, dopamine, GABA and glutamate was shown for the CO_2 extract (38.8% hyperforin) and for pure hyperforin. IC_{50} values of the methanolic extract (1.5% hyperforin) were about 10 times higher than those of the CO_2 extract, indicating that other constituents besides hyperforin must cause reuptake inhibition [115].

A hydromethanolic extract of St. John's wort (hyperforin content < 5%) inhibited the accumulation of [^{3}H]serotonin (5-HT) in rat brain cortical synaptosomes with an IC_{50} of 7.9 µg/ml, compared to 1.8 µg/ml for pure hyperforin. Thus the activity of the extract cannot be explained solely by its hyperforin content. The same extract at 3-10 µg/ml and hyperforin at 0.3-1 µg/ml induced marked tritium release from synaptosomes preloaded with [^{3}H]5-HT. This indicates reserpine-like properties and excludes marked serotonin uptake inhibition as the mode of action of a hydromethanolic extract of St. John's wort [116].

A hydromethanolic extract from St. John's wort (0.3% hypericin, 4.9% hyperforin) inhibited serotonin and

norepinephrine uptake in astrocytes in a dose-dependent manner with IC_{50} values of 25 µg/ml and 10 µg/ml respectively [117]. A hydromethanolic extract from St. John's wort showed only weak inhibitory activity on MAO-A and MAO-B, but effectively inhibited synaptosomal uptake of serotonin, dopamine and norepinephrine with IC_{50} values of 2.43, 0.85 and 4.47 µg/ml respectively [118].

Hyperforin inhibited the reuptake of serotonin, dopamine, noradrenaline and GABA with IC_{50} values of 0.05-0.1 µg/ml and of glutamate with an IC_{50} of 0.5 µg/ml in rat synaptosomal preparations [115]. Elevation of free intracellular Na^+ seems to be responsible for the reuptake inhibition of hyperforin [119]. Hyperforin was tested on voltage- and ligand-gated ionic conductances to measure neuronal responses on stimulation of NMDA (N-methyl-D-aspartate) or AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors. At concentrations between 3 and 100 µM antagonistic effects on the NMDA receptor and on responses mediated by AMPA or GABA were seen [120]. Hyperforin was found to non-competitively inhibit the synaptosomal uptake of ^{3}H-glutamate and ^{3}H-GABA [121].

Immunological parameters

A hydromethanolic extract of St. John's wort caused pronounced suppression of interleukin-6 release in human blood samples after stimulation with phytohaemagglutinin [122]. Hypericin in the micromolar range inhibited phorbol myristate acetate (PMA)- and TNF-α-induced activation of NF-κB, whereas hyperforin or a St. John's wort extract containing 0.15% hypericin and 5% hyperforin were inactive [123]. The myeloperoxidase-catalyzed dimerization of enkephalins was inhibited by St. John's wort extracts [124].

Mononuclear cells from healthy donors were incubated overnight with a hydromethanolic extract from St. John's wort or serotonergic antidepressant drugs (selective serotonin reuptake inhibitors, SSRIs). Cells were tested for natural killer cell activity, since serotonergic pharmacotherapy is associated with an increase in natural killer cell activity. The extract was tested at very low concentrations (0.5-20 ng/ml), paroxetine at 120 ng/ml and norfluoxetine at 100 ng/ml. In contrast to the SSRIs, St. John's wort extract failed to enhance natural killer cell activity at the concentrations studied [125].

In vivo experiments

Behavioural studies

Hydroethanolic preparations of St. John's wort containing known amounts of hypericin (equivalent to 2-12 mg/kg, administered orally) showed CNS activities in mice which can be interpreted as an antidepressant effect. Aggressive behaviour was significantly reduced after 3 weeks of daily oral treatment with extract equivalent to 6 or 12 mg/kg of hypericin. Physical activity was enhanced and no undesired anticholinergic effect was found. A significant increase in physical activity was also observed after intraperitoneal administration of pure hypericin at 20 mg/kg. The same extract significantly increased ethanol-induced sleeping time after oral doses equivalent to 2.4 or 6 mg/kg of hypericin. Oral administration of the extract to mice at doses equivalent to 1, 2 or 3 mg/kg (but not 6 mg/kg) of hypericin resulted in weak reserpine antagonism, indicated by reductions in reserpine-induced hypothermia [126].

A hydromethanolic extract of St. John's wort (0.24-0.32% total hypericin) was studied in a rat model for antidepressant activity, the Porsolt forced-swimming test. At doses of 125-1000 mg/kg body weight the extract significantly reduced the duration of immobility; lower and higher doses were ineffective, indicating a U-shaped dose-activity relationship. As motor activity was not increased in this dose range and the activity was confirmed by repeated application, these findings indicate antidepressant activity. The extract also reduced ketamine-induced sleeping time and increased body temperature in mice. The effects of this extract on immobility time in the Porsolt test, on sleeping time and on body temperature were eliminated by dopamine D_2 antagonists, indicating dopaminergic activity of the extract [127].

After acute oral administration to rats at 250-500 mg/kg, a St. John's wort extract enriched in flavonoids (0.3% hypericin, 4.5% hyperforin and 50% flavonoids) significantly and dose-dependently decreased immobility time in the Porsolt forced-swimming test ($p<0.001$ compared to saline). Compared to the effect of the extract alone at 500 mg/kg, concomitant treatment of the rats with sulpiride (a dopamine receptor antagonist) or metergoline (a serotonin receptor antagonist) or 6-hydroxydopamine (which destroys noradrenaline-containing neurons) significantly increased the period of immobility by 22-57%, the largest effect being with metergoline (57%; $p<0.001$). The results indicated that the neurotransmitters studied could be involved in the anti-immobility effects of St. John's wort and suggest that its antidepressant action is probably mediated by serotonergic, noradrenergic and dopaminergic system activation; an increase in serotonergic tone is probably predominant [128].

A hydroethanolic extract proved to be active in three animal models of depression: an acute form of escape deficit (ED), a subacute form of ED and a model of anhedonia. Oral dosages between 250 and 1000 mg/kg body weight were used. The activity of the extract was clearly reduced by antagonists at the dopamine D_1 receptor or the serotonin 5-HT_{1A} receptor [129].

Adaptive changes
Following subacute treatment of rats with 240 mg/kg of a methanolic St. John's wort extract, β-adrenoreceptor down-regulation was observed in the frontal cortex, a common characteristic finding for many antidepressant drugs. The simultaneous up-regulation of 5-HT_2 receptors observed was in contrast to the 5-HT_2 down-regulation observed with many classic antidepressants [118].

Activity of constituents
Chromatographic fractionation of a methanolic St. John's wort extract yielded two fractions with high activity in the Porsolt test, one containing mainly flavonoids, the other naphthodianthrones. Pure hypericin was inactive in the Porsolt test at a dose of 0.8 mg/kg body weight, but procyanidins present in the naphthodianthrones fraction, namely procyanidin B2, markedly increased solubility as well as bioactivity; in combination with B2 even 0.009 mg/kg hypericin was significantly active. Comparable results were obtained with pseudohypericin. Activity due to procyanidin B2 alone could be excluded [130].

In addition to hypericins, a flavonoid fraction from a methanolic extract of St. John's wort and the isolated flavonoids hyperoside, isoquercitrin and miquelianin (0.6 mg/kg) showed significant activity in the Porsolt test after acute and subacute administration [131].

A supercritical carbon dioxide extract from St. John's wort (38.8% hyperforin) was compared to an ethanolic extract (4.5% hyperforin) for activity in the Porsolt test and the learned helplessness paradigm in rats. Both extracts were active, the effect of 5, 15 and 30 mg/kg body weight of the CO_2 extract being comparable to that of 50, 150 or 300 mg/kg of the ethanolic extract [115]. A hydromethanolic and a hydroethanolic extract were compared for activity in the Porsolt test after acute administration. Both extracts, injected intraperitoneally, reduced immobility time in the dose range of 5-40 mg/kg body weight. The effect was more pronounced after one week of pretreatment [132]. After 3 days of pre-treatment, a 50% ethanolic extract of St. John's wort, administered orally at 100 and 200 mg/kg, was as effective as an antidepressant drug (imipramine at 15 mg/kg intraperitoneally) in the Porsolt test, the learned helplessness paradigm and the tail suspension test [133].

Biochemical findings in vivo

St. John's wort extracts
After acute oral administration (24 hours and 1 hour before testing) of a methanolic St. John's wort extract (62.5-500 mg/kg; 0.3% hypericin, 6% flavonoids) or an extract enriched in flavonoids (62.5-500 mg/kg; 0.3% hypericin, 50% flavonoids) their effects on levels of tryptophan, serotonin (5-HT), 5-hydroxyindolacetic acid (5-HIAA), noradrenaline and dopamine in the cortex, diencephalon and brainstem of rats were evaluated. Results with respect to 5-HT turnover were compared with the effects of fluoxetine (10-80 mg/kg), a selective serotonin reuptake inhibitor with antidepressant activity. The two St. John's wort extracts and fluoxetine increased 5-HT levels in the cortex. The flavonoid-enriched extract increased 5-HT and 5-HIAA levels in the diencephalon, and 5-HT and noradrenaline levels in the brainstem. Both the St. John's wort extracts increased noradrenaline and dopamine levels in the diencephalon [134]. Comparable results for the flavonoid-enriched extract were obtained in a subsequent study using similar techniques; it did not modify tryptophan content, but significantly enhanced 5-HT and 5-HIAA levels in the cortex, diencephalon and brainstem at 125-500 mg/kg, and increased noradrenaline and dopamine in the diencephalon and noradrenaline in the brainstem at 250-500 mg/kg [128].

Rats were treated orally with 300 mg/kg of a hydromethanolic extract of St. John's wort three times (23.5 hours, 5 hours and 1 hour before the experimental period), then tested in the Porsolt forced swimming test; the extract significantly reduced immobility time ($p<0.01$), confirming antidepressant-like properties. However, in rats treated with the same extract by the same schedule, no significant changes in monoamine levels were detected in cortical or hippocampal brain regions 1 hour and 24 hours after the last dose [116].

Following long term administration (26 weeks) of a hydromethanolic extract of St. John's wort to rats at 2700 mg/kg/day, the number of serotonin receptors (5-HT_{1A} and 5-HT_{2A}) in the brain had increased by 50%; the affinity was unaltered [135]. After acute and repeated treatment of mice with as little as 10 mg/kg of St. John's wort extract, the levels of serotonin and the serotonin metabolite 5-hydroxyindolacetic acid were increased in hypothalamus and hippocampus indicating increased turnover; 5-hydroxyindolacetic acid levels were also increased in other regions of the brain [136].

Effects on the levels of serotonin (5-HT), norepinephrine, dopamine and their metabolites in the hypothalamus and hippocampus of rat brain were investigated after short-term (2 weeks) and long-term (8 weeks) daily oral administration to rats of the tricyclic antidepressant imipramine (15 mg/kg) or St. John's wort extract (500 mg/kg) or hypericin (0.2 mg/kg). All three treatments significantly increased 5-HT levels in the hypothalamus ($p<0.05$) after 8 weeks, but not after 2 weeks; levels in the hippocampus were unchanged. 5-HT turnover (the ratio of 5-HIAA to 5-HT) was significantly reduced in both brain regions (both $p<0.05$) after 8 weeks of treatment with the St. John's wort extract (but not with hypericin); imipramine reduced 5-HT turnover only in the hippocampus. Consistent changes in catecholamine levels were

only detected in hypothalamic tissues after long-term treatment. Comparable to imipramine, St. John's wort extract and hypericin significantly decreased 3,4-dihydroxyphenylacetic acid and homovanillic acid levels in the hypothalamus ($p<0.01$). The data showed that long-term, but not short-term, administration of St. John's wort and its active constituent hypericin modified levels of neurotransmitters in brain regions involved in the pathophysiology of depression [137].

Activity of constituents

The effects of an ethanolic extract containing 4.5% of hyperforin (50, 150 and 300 mg/kg) were compared to those of a supercritical carbon dioxide extract with 38.8% of hyperforin but devoid of hypericins (5, 15 and 30 mg/kg) after oral administration to rodents on three consecutive days. Differences in activity became obvious. The ethanolic extract increased dopaminergic behavioural responses, e.g. in the DOPA-potentiation test and the apomorphine-induced stereotypy, whereas the CO_2 extract exerted more pronounced serotoninergic responses, e.g. 5-hydroxytryptophan-induced increase in the number of head twitches [138].

Microdialysis probes were implanted into the left nucleus accumbens, in the left striatum or in the ventral hippocampus of rats. After oral administration of 1 mg/kg of a CO_2 extract a slight but significant increase of dopamine outflow was observed in both the nucleus accumbens and the striatum, whereas serotonin release remained unchanged [139].

Using the push-pull superfusion technique, neurotransmitter concentrations in the locus coeruleus of rats were studied after intraperitoneal injection of hyperforin at 10 mg/kg. Extracellular concentrations of dopamine, noradrenaline, serotonin and glutamate had increased, whereas 5-HIAA, GABA, taurine, aspartate, serine and arginine remained unchanged [140].

A methanolic (4.67% hyperforin) and a CO_2 (30.14% hyperforin) extract of St. John's wort, administered orally in amounts providing equal hyperforin content, were compared for their effects on the "tele-stereo-EEG" of freely moving rats. At first both extracts produced increases in the alpha-1 band of the striatum; later only the methanolic extract caused an increase in delta activity [141].

Using the resources of the National Institute of Mental Health Psychoactive Drug Screening Program (USA), the *in vitro* pharmacology of various pure constituents of St. John's wort (hypericin, pseudohypericin, hyperforin, quercetin, isoquercitrin, quercitrin, miquelianin, rutin, hyperoside and amentoflavone) has been characterized at 42 biogenic amine receptors and transporters using radioligand binding assays. The compounds were screened for activity at G-protein-coupled receptors (GPCRs) including 5-HT, adrenergic, opioid, histamine, metabotropic glutamate and muscarinic acetylcholine, and ligand-gated ion channels including $GABA_{A/B}$ receptors as well as various neurotransmitter transporters. The data clearly demonstrated that some of the investigated compounds showed unanticipated binding inhibition in several receptor assays, whereas most were inactive. The most potent binding activities were observed for amentoflavone, hypericin and pseudohypericin; these showed distinct spectra of activity across receptor assays such as dopaminergic, adrenergic or opioid systems. In contrast, hyperforin and the flavonoids showed only relatively weak binding inhibition in the same systems. Amentoflavone significantly inhibited binding at serotonin ($5\text{-}HT_{1D}$, $5\text{-}HT_{2C}$), dopamine D3, δ-opioid and benzodiazepine receptors. Hypericin and pseudohypericin had significant activity at dopamine D3 and D4 receptors, and hypericin (but not pseudohypericin) at β-adrenergic receptors. With the exception of dopamine D1 and D5 receptors, hyperforin was less active than the other tested constituents on all screened receptors. Taken together, these data revealed novel interactions of St. John's wort constituents with a number of GPCRs, but further studies are necessary to establish their *in vivo* pharmacological relevance to therapeutic effects [142].

In summary, *in vitro* and *in vivo* experiments have demonstrated relevant activity of naphthodianthrones, hyperforin and flavonoids. Although the mode of action of St. John's wort extracts is still under discussion, activity comparable to that of synthetic antidepressants has been observed in diverse test systems.

Effects on ethanol preference

Ethanol preference and ethanol intake in two strains of alcohol-preferring rats were significantly reduced by a methanolic extract containing 0.22% of naphthodianthrones and 4.05% of hyperforin. No tolerance developed during 2 weeks of oral treatment with 400 mg/kg/day of the extract; the reduction in ethanol preference remained unchanged [143]. A hydroethanolic extract of St. John's wort (0.3% hypericin, 3.8% hyperforin), injected intraperitoneally at 10-40 mg/kg, reduced both ethanol intake and ethanol preference [132]. The same St. John's wort extract, administered intragastrically at 250 mg/kg, was active in the Porsolt test and reduced ethanol intake in alcohol-preferring rats. Since the anti-immobility effect was abolished by σ-receptor blockade and diminished in the presence of experimentally lowered serotonin levels, whereas the ethanol intake remained unchanged under these conditions, both activities seem to be caused by other mechanisms [144].

Other pharmacological effects

Externally applied St. John's wort preparations have been reported to have anti-inflammatory and anti-

bacterial effects [145].The antibiotic effect has been attributed to the presence of hyperforin [146]. A hydroethanolic extract reduced croton oil-induced ear oedema in rodents by 50% ($p<0.05$); from fractionation experiments it was concluded that the anti-inflammatory principle is concentrated in the lipophilic fractions [147].

In vitro antibacterial activity of hyperforin against Gram-positive bacteria and multiresistant *Staphylococcus aureus* has been reported [148]. However, this activity was obvious only at high concentrations [149,150].

Strong antiviral effects of hypericin have been demonstrated *in vitro* [151-158]. Antiviral activity of hypericin and pseudohypericin has also been demonstrated *in vivo*; a single intravenous 50 µg dose completely inhibited splenomegaly (enlargement of the spleen) induced in mice by Friend leukaemia virus (FV), an aggressive retrovirus [156], and a single intravenous 150 µg dose enabled survival of FV-inoculated mice for at least 240 days compared to 23 days for untreated FV-inoculated animals, hypericin being the more potent antiretroviral agent [155].

A dry ethanolic extract of St. John's wort showed strong antioxidant activity *in vitro* and, when administered orally to rats at 250 mg/kg/day, significantly reduced immobilization stress-induced lipid peroxidation (measured *ex vivo* in liver homogenate; 42% protection after 30 days, $p<0.001$) [159].

Clinical studies

The efficacy and safety of standardized hydroalcoholic St. John's Wort extracts have been assessed in 31 controlled, double-blind [24-47,50,53,56-58,92,93] and 2 open [51,52] studies, involving more than 3900 patients and 13 different preparations; drug monitoring studies and numerous case reports have involved a further 10,000 patients [80-91,160]. The major indication in most of the studies was mild or mild to moderate depressive disorders. Three studies were designed for treatment of severe depressive disorders [56-58]. A significant improvement in main symptoms (mood, loss of interest and activity) and other symptoms of the depressive syndrome (sleep, concentration, somatic complaints) has been demonstrated in many of these trials. The activity was studied against placebo and against different antidepressants (amitriptyline, imipramine, maprotiline, fluoxetine), and in two studies simultaneously against placebo and imipramine or placebo and sertraline. Three meta-analyses [161-163] and six systematic reviews [164-169] of clinical trials with different selection criteria, have confirmed the efficacy of various St. John's wort extracts in mild to moderate depression, but not in severe depression.

The studies summarized below are grouped according to the preparations tested. Essential similarity is assumed for hydroalcoholic dry extracts within the range 60% ethanol to 80% methanol on the basis of their similar active constituents profiles [170]. An overview of the controlled studies is given in Table 1.

ABBREVIATIONS used in the summaries of clinical studies

CGI	Clinical Global Impressions scale [172]
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised [173]
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th ed. [174]
HAMA	Hamilton Anxiety Scale [175]
HAMD	Hamilton Depression Scale [176,177]
ICD-09	International Classification of Diseases, Ninth Revision: Mental Disorders [178]
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision [48,49].

TABLE 1: OVERVIEW OF CONTROLLED CLINICAL STUDIES

First Author Year Reference number	Number of patients	Type of preparation Extraction solvent	Daily dosage	Reference therapy	Duration (days)	Mean intial HAMD	Severity of depression
Sommer 1994 [29]	105	Dry extract, 80% methanol	3 × 300 mg*	Placebo	28	15.8	Mild
Hänsgen 1996 [38]	102	Dry extract, 80% methanol	3 × 300 mg	Placebo	42	20.7	Mild to moderate
Häring 1996 [31]	28	Dry extract, 80% methanol	3 × 300 mg	Placebo	Not stated	Not stated	Mild
Martinez 1994 [51]	20	Dry extract, 80% methanol	3 × 300 mg	Phototherapy	28	21.3	Mild to moderate
Hübner 1994 [30]	40	Dry extract, 80% methanol	3 × 300 mg	Placebo	28	12.5	Mild
Harrer 1994 [39]	102	Dry extract, 80% methanol	3 × 300 mg	Maprotiline	28	21.0	Mild to moderate
Vorbach 1994 [40]	135	Dry extract, 80% methanol	3 × 300 mg	Imipramine	42	19.8	Mild to moderate
Wheatley 1997 [41]	165	Dry extract, 80% methanol	3 × 300 mg	Amitriptyline	42	20.7	Mild to moderate
Vorbach 1997 [58]	209	Dry extract, 80% methanol	3 × 600 mg	Imipramine	42	25.7	Severe
Shelton 2001 [56]	200	Dry extract, 80% methanol	3 × 300-400 mg	Placebo	56	22.5	Severe
Davidson 2002 [57]	340	Dry extract, 80% methanol	3 × 300-500 mg	Sertraline; Placebo	56	22.8	Moderate to severe
Lehrl 1993 [50]	50	Dry extract, 80% methanol	3 × 300 mg*	Placebo	28	22.7	Mild to moderate
Halama 1991 [34]	50	Dry extract, 80% methanol	3 × 300 mg*	Placebo	28	18.3	Mild to moderate
Laakmann 1998 [35]	147	Dry extract, 60% ethanol	3 × 300 mg	Different St. John's wort extract; Placebo	42	20.8	Mild to moderate
Kalb 2001 [36]	72	Dry extract, 60% ethanol	3 × 300 mg	Placebo	42	19.9	Mild to moderate
Philipp 1999 [37]	263	Dry extract, 60% ethanol	3 × 350 mg	Imipramine; Placebo	56	22.6	Moderate
Harrer 1999 [28]	149	Dry extract, 60% ethanol	2 × 400 mg	Fluoxetine	42	16.9	Mild
Witte 1995 [42]	97	Dry extract, 50% ethanol	2 × 100-120 mg	Placebo	42	23.6	Moderate
Schrader 1998 [43]	162	Dry extract, 50% ethanol	2 × 250 mg	Placebo	42	19.5	Mild to moderate
Schrader 2000 [44]	240	Dry extract, 50% ethanol	2 × 250 mg	Fluoxetine	42	19.6	Mild to moderate
Woelk 2000 [45]	324	Dry extract, 50% ethanol	2 × 250 mg	Imipramine	42	22.3	Mild to moderate
Warnecke 1986 [52]	60	Tincture, 50% ethanol	3 × 1.5 ml	Diazepam	94	---	Mild
Hoffmann 1979 [24]	60	Tincture, 50% ethanol	3 × 1.5 ml	Placebo	42	---	Mild
Schlich 1987 [25]	49	Tincture, 49% ethanol	3 × 1 ml	Placebo	28	23.5	Mild
Schmidt 1989 [32]	40	Tincture, 49% ethanol	3 × 1.5 ml	Placebo	28	29.4	Mild to moderate
Harrer 1991 [33]	120	Tincture, 49% ethanol	3 × 1.5 ml	Placebo	42	21.3	Mild to moderate
Quandt 1993 [27]	88	Tincture, 49% ethanol	3 × 1.5 ml	Placebo	28	17.6	Mild
Werth 1989 [53]	30	Tincture, 49% ethanol	3 × 1.5 ml	Imipramine	23	---	Mild
Kugler 1990 [26]	80	Tincture, 49% ethanol	3 × 1.5 ml	Bromazepam	28	---	Mild
Lenoir 1999 [46]	348	Dry ethanolic extract fromfresh shoot tips	3 × 60 mg	Different dose levels of same extract	42	16.5	Mild
Engesser 1996 [47]	19	Tea preparation	Not stated	Milfoil tea	14	---	Mild

*Daily dosage was up to 50% lower due to up to 50% of excipients in the amount stated [171].

*Clinical studies and drug surveillance studies in **mild to moderate depression** performed with hydro-methanolic extracts (80% methanol)*

In 102 outpatients (mean age 52 years) with depression of mild or moderate severity (DSM-III-R: 296.2, 296.3), 3 × 300 mg of a St. John's wort extract (4-7:1, methanol 80%) daily over a period of 4 weeks was compared to placebo. Average HAMD scores at baseline were 21 in the verum group and 20.4 in the placebo group. After 4 weeks the average score was significantly lower ($p<0.001$) in the verum group (8.9) than in the placebo group (14.4). Similar results were obtained on the von Zerssen self-rating depression scale ($p<0.001$). Responder rates, defined as at least 50% reduction in initial HAMD score or a final score of less than 10, were 70% under verum and 24% under placebo ($p<0.001$). This randomized, double-blind period was followed by a further 2-week period during which all patients were treated openly with the St. John's wort extract; HAMD scores decreased further, to 6 in the former verum group and more substantially to 8.7 in the former placebo group [38].

In a placebo-controlled, randomized, double-blind study, 40 patients (mean age 51 years) suffering from mild depression with somatic symptoms (ICD-09: 300.4, neurotic depression; 309.0, brief depressive reaction) were treated with 3 × 300 mg of a St. John's wort extract (4-7:1, methanol 80%) or placebo daily for 4 weeks. Initial mean HAMD scores of 12.5 dropped to 5.5 in the St. John's wort group compared to 10.8 in the placebo group ($p<0.05$). Patients in the placebo group had an improved score of 9 after 2 weeks, but this subsequently increased. Responder rates, defined by an endpoint score of less than 10 or a drop of at least 50% from baseline value, were 70% and 47% respectively [30].

In a prospective, placebo-controlled, double-blind pilot study, 28 outpatients undergoing a chemotherapy regimen for solid tumours were randomly assigned to 3 × 300 mg of a St. John's wort extract (4-7:1, methanol 80%) or placebo daily for 2-3 chemotherapy treatment cycles. Although the average HAMD score in the St. John's wort group showed a slight trend towards improvement, results from the two treatment groups were comparable with respect to a quality of life analysis, the von Zerssen self-rating depression scale and the CGI scale [31].

In an open, randomized, single-blind study involving patients (mean age 46 years) suffering from seasonal affective disorder (major depression with a seasonal pattern diagnosed in accordance with DSM-III-R), daily treatment for 4 weeks with 3 × 300 mg of a St. John's wort extract (4-7:1, methanol 80%) was combined with phototherapy: either bright light (3000 lux; $n = 10$) or dim light (< 300 lux, $n = 10$) for 2 hours per day. Average HAMD scores at baseline (21.9 in the bright light group and 20.6 in the dim light group) decreased significantly in both groups, to 6.1 and 8.2 respectively ($p<0.001$). Due to the small sample size, the difference between groups was not statistically significant [51].

In a 4-week randomized, double-blind, comparative study (no placebo group), the efficacy and tolerability of 3 × 300 mg daily of a St. John's wort extract (4-7:1, methanol 80%) was compared with 3 × 75 mg daily of the antidepressant maprotiline in 102 patients of mean age 46 years with a diagnosis of moderate depression (ICD-10: F 32.1). Initial HAMD scores fell from 20.5 to 12.2 in the St. John's wort group and from 21.5 to 10.5 in the maprotiline group. Responder rates, defined by an endpoint score of less than 10 or a drop of at least 50% from the baseline, were 61% and 67% respectively. Overall, there were no significant differences between groups [39].

In a 6-week multicentre, randomized, double-blind, comparative study without placebo control, 3 × 300 mg daily of a St. John's wort extract (4-7:1, methanol 80%) was compared to 3 × 25 mg daily of the antidepressant imipramine. The 135 patients (mean age 53 years) had diagnoses in accordance with DSM-III-R criteria of depression with a single episode (296.2), several episodes (296.3), depressive neurosis (300.4) or adjustment disorder with depressed mood (309.0). The main outcome criteria were HAMD scores, the von Zerssen depression scale (D-S) and CGI. Initial HAMD scores declined from 20.2 to 8.8 in the St. John's wort group and from 19.4 to 10.7 points in the imipramine group. Group comparisons showed no significant differences. Analysis of D-S and CGI patterns in the two groups also revealed comparable results. Subgroups with initial HAMD scores of 21 or more performed significantly better ($p<0.05$) after St. John's wort extract ($n = 26$) than after imipramine ($n = 25$) with regard to HAMD score and CGI [40].

In a 6-week multicentre, randomized, double-blind, comparative study without placebo group, 165 mildly to moderately depressed outpatients (mean age 40 years) diagnosed in accordance with DSM-IV were treated with 3 × 300 mg daily of a St. John's wort extract (4-7:1, methanol 80%) or 3 × 25 mg daily of the sedative tricyclic antidepressant amitriptyline. Initial HAMD scores were between 17 and 24 (mean 20.7). No statistically significant difference between groups was observed with respect to the primary outcome parameter, the response rate (defined as an endpoint HAMD score of less than 10, or a drop of at least 50% from baseline values). 59.7% of patients in the St. John's wort group were classified as responders compared to 77.8% in the amitriptyline group ($p = 0.064$). HAMD scores had dropped to 10 in the St. John's wort group and to 6 in the amitriptyline group after 6 weeks, a difference significantly in favour of amitriptyline ($p<0.05$). Analysis of another secondary

efficacy parameter, the Montgomery-Asberg rating scale for depression (MADRS), also favoured amitriptyline ($p<0.05$) [41].

In a multicentre drug surveillance study 3250 patients (mean age 51 years), of whom 49% had mild, 46% moderate and 3% severe depressive symptoms, were monitored for 4 weeks while taking 3 × 300 mg daily of a St. John's wort extract (4-7:1, methanol 80%). Scores using the von Zerssen Depression Scale dropped from 23.2 to 11.8 over the 4-week period. At the end of treatment, 82% of patients were assessed as improved or symptom-free by the physicians and 79% by the patients. Although efficacy was only slightly dependent on age, the therapeutic effect was better in patients younger than 50 years. Cases with mild or moderate depression responded to treatment to the same extent, while severe cases improved somewhat less [85].

In a multicentre drug surveillance study, 1060 patients (mean age 51 years) with mild to moderate depressive symptoms were assessed over a 4-week period of treatment with 3 × 300 mg daily of a St. John's wort extract (4-7:1, methanol 80%). By the end of the study, the average HAMD score had dropped from 18.4 to 5.4 and the von Zerssen self-rating depression score from 21.1 to 7.3. According to CGI evaluation, 66% of patients had improved and 27% were symptom-free after 4 weeks [86].

In a 5-week drug monitoring study, the efficacy of a St. John's wort extract (3 × 135-225 mg of extract, providing 900 µg of total hypericin daily) was assessed in 114 patients (mean age 48 years) with mild depressive symptoms. The treatment was evaluated by the von Zerssen self-rating depression scale (D-S), the distribution of characteristic psychic and somatic symptoms, and a global rating by the physician. From an initial 21 the D-S score dropped to 15 after 2 weeks and to 2 after 5 weeks. The physicians rated 39% of patients as improved and 35% as symptom-free after 5 weeks [87].

In a 12-week drug-monitoring study the efficacy of a St. John's wort extract (3 × 135-225 mg of extract, providing 900 µg of total hypericin daily) was assessed in 111 women (mean age 52 years) with menopausal symptoms. Women who had received hormone replacement therapy with oestrogens or oestrogen-progestogen combinations were excluded from the study. Treatment was evaluated by the menopause rating scale (MRS), a questionnaire for assessing sexuality, and the CGI scale. Climacteric complaints diminished or disappeared completely in 79% of the women as rated by the physician (CGI rating better or symptom-free). Sexual well-being was favourably affected in approximately 60% of the women. The average MRS total score dropped from an initial 63.4 (corresponding to a marked intensity of symptoms) to 23.5 (slight intensity) after 12 weeks [88].

In another drug-surveillance study 647 patients with mild to moderate depression were treated daily for 6 weeks with 3 × 300 mg of a St. John's wort extract (4-7:1, methanol 80%). In 75% of the patients the condition improved. The von Zerssen self-rating depression score dropped from 19.8-21.2 at baseline to 8.1-9.3 after 6 weeks. In patients older than 65 years the condition improved at a somewhat slower rate but the severity of depression did not appear to affect the outcome [89].

In a multicentre drug monitoring study with 1606 patients (mean age 52 years) suffering from typical depressive symptoms such as low mood, loss of interest and energy, and disturbed sleep, more than 90% were treated 2-3 times daily with 300 mg of a St. John's wort extract (4-7:1, methanol 80%; standardized to 900 µg of total hypericin). After a mean treatment period of 5 weeks, the intensity of dominating symptoms was markedly reduced. The efficacy was rated as very good or good by 81% of the physicians and 76% of patients. No age-dependent correlation of efficacy was reported [90].

Older clinical studies in ***mild to moderate depression*** *performed with hydromethanolic extracts (80% methanol) at lower daily dosages (see the footnote to Table 1).*

In a multicentre, randomized, double-blind study, 105 patients (mean age 45 years) with depressive symptoms (ICD-09: 300.4, neurotic depression; or 309.0, brief depressive reaction) were allocated to 3 × 300 mg of a St. John's wort extract (4-7:1, 80% methanol), or placebo daily for 4 weeks. By the end of the study initial HAMD scores (15.8 points in both groups) had dropped to 7.2 in the St. John's wort group and 11.3 in the placebo group, a statistically significant difference ($p<0.01$). Responder rates, defined by a total endpoint score of less than 10 or a drop of at least 50% from baseline values, were 67% and 28% respectively [29].

In a multicentre, placebo-controlled study, 50 patients (mean age 49 years) with depressive symptoms (ICD-09: 300.4 and 309.0) were treated daily for 4 weeks with either 3 × 300 mg of a St. John's wort extract (4-7:1, methanol 80%) or placebo. HAMD scores improved from 23.7 to 17.4 in the verum group and from 21.6 to 16.8 in the placebo group, the difference not being significant. In an evaluation of cognitive performance using the short test for general basic capacities of information processing (KAI), patients on St. John's wort extract showed slightly better improvement than those on placebo ($p<0.1$) [50].

In a randomized, placebo-controlled study involving 50 patients suffering from psychovegetative depressive

symptoms (ICD-09: neurotic depression or depressive mood of short duration), 3 × 300 mg daily, in some cases reduced to 2 × 300 mg after week 2, of a St. John's wort extract (4-7:1, methanol 80%) led to a substantial improvement after 4 weeks of therapy. A reduction of at least half from their initial HAMD total score or a score below 10 after 4 weeks was achieved by 50% of patients in the St. John's wort group ($p<0.01$), but by none in the placebo group. Other parameters, such as the von Zerssen Complaint Score and CGI score, showed similar differences between verum and placebo [34].

*Clinical studies in **major depression** or **severe, recurrent depressive episodes** in accordance with ICD-10 F33.2, performed with hydromethanolic extracts (80% methanol)*

Two hundred adult outpatients (mean age 42 years) with a DSM-IV diagnosis of major depression and an initial HAMD total score of at least 20 participated in a randomized, double-blind, placebo-controlled study conducted in 11 academic medical centres in the USA. The patients were suffering from recurrent depression (64%) or a single episode of depression (35%) and around 40% were melancholic. The duration of the current major depressive disorder was more than 2 years on average and onset of the initial major depressive disorder was more than 10 years ago. Initial HAMD total scores were about 22.5 (estimated from a graph). After a 1-week, single-blind run-in with placebo, patients were treated for 8 weeks with either a St. John's wort extract (4-7:1, methanol 80%), 3 × 300 mg daily for 4 weeks, increased thereafter to 4 × 300 mg daily in the absence of adequate response, or placebo. The primary outcome measure was the rate of change in HAMD score over the treatment period. Random coefficient analyses for the HAMD showed significant effects for time ($p<0.001$) but not for treatment ($p = 0.16$) or time-by-treatment interaction ($p = 0.58$). Similar results were obtained for the secondary outcome measures, HAMA and CGI. 26.5% of patients in the St. John's wort group and 18.6% in the placebo group were responders (defined a priori as HAMD score of 12 or less and CGI Intensity score of 1 or 2); these proportions were not statistically different. A significantly higher ($p = 0.02$) remission rate (remission being defined as an endpoint HAMD score of 7 or less and CGI-I score of 1 or 2) was estimated for St. John's wort (14.3%) than for placebo (4.9%), but both rates were low. In this study St. John's wort was not effective for the treatment of rather severe depression [56].

In a multicentre, randomized, placebo-controlled, double-blind, three-armed study, 340 outpatients (mean age 42 years) with major depression of moderate severity (DSM-IV: maximum score of 60 on Global Assessment of Functioning) and a baseline HAMD score of at least 20 were assigned to one of three treatments daily for 8 weeks: 3 × 300 mg of a St. John's wort extract standardized to 0.12-0.28% hypericin and containing 3.1% of hyperforin (although not stated, the identified extract is assumed to have had a drug-to-extract ratio of 4-7:1, extracted with methanol 80%) or 50 mg of sertraline (divided into 3 doses) or placebo. Daily dosage could be increased after week 3 to 1500 mg of St. John's wort extract or to 100 mg of sertraline if warranted; the mean highest daily doses prescribed during the 8-week period were 1299 mg of St. John's wort extract, 75 mg of sertraline or placebo equivalent. With respect to the two primary efficacy parameters, change from HAMD baseline and full response rates, defined as an endpoint HAMD score of 8 or less and a CGI-I score of 1 (very much improved) or 2 (much improved), neither St. John's wort extract nor sertraline were significantly different from placebo over the 8-week period. Mean reductions in HAMD scores were 8.7 for St. John's wort extract, 10.5 for sertraline and 9.2 for placebo. Full response rates were 23.9% for St. John's wort extract, 24.8% for sertraline and 31.9% for placebo. The results did not therefore support efficacy of St. John's wort extract in moderately severe major depression [57].

In a 6-week multicentre, randomized, double-blind comparative trial without placebo group, 209 patients with a mean age of 49 years (38 hospitalized and 171 outpatients treated by psychiatrists) were allocated to 3 × 600 mg daily of a St. John's wort extract (4-7:1, methanol 80%) or 3 × 50 mg daily of imipramine. The inclusion criteria were defined in accordance with ICD-10 F33.2: a severe episode of a major depressive disorder, recurrent, without psychotic symptoms. The primary target parameter, mean HAMD scores, dropped from 25.3 to 14.4 points in the St. John's wort group and from 26.1 to 13.4 points in the imipramine group, the difference between groups being significant in favour of imipramine ($p = 0.021$). Equivalence of the two treatments could be shown with respect to response rates, defined as the percentage of patients showing a reduction of at least 50% in HAMD total score over the study period: 35.3% in the St. John's wort group, 41.2% in the imipramine group [58].

*Clinical studies and drug surveillance studies in **mild to moderate depression** performed with hydro-ethanolic extracts (ethanol 60%)*

In a multicentre, randomized, double-blind, placebo-controlled study, 147 outpatients (mean age 49 years) suffering from depression of mild or moderate severity in accordance with DSM-IV criteria, and with initial HAMD scores of at least 17, were treated daily for 6 weeks with placebo or 3 × 300 mg of one of two St. John's wort extracts (4:1, no data on naphthodianthrones): one extract contained 5% hyperforin, the other 0.5% hyperforin. The average HAMD score at baseline was 20.8. At the end of the study, the extract group receiving the 10-fold higher content of

hyperforin had the largest reduction in HAMD score from baseline (10.3 ± 4.6), followed by the extract group with a lower content of hyperforin (8.5 ± 6.1) then the placebo group (7.9 ± 5.2). After 6 weeks the proportions of responders (defined as a reduction of at least 50% from initial HAMD scores) were 49% in the 5% hyperforin group, 39% in the 0.5% hyperforin group and 33% in the placebo group. HAMD score reduction was statistically superior to placebo only in the 5% hyperforin group ($p = 0.004$, Mann-Whitney U-test). Results were similar for other outcome measures (CGI scale and von Zerssen self-rating depression scale). The important contribution of hyperforin to the antidepressant activity of St. John's wort was deduced from this study [35].

Another 6-week multicentre, randomized, double-blind, placebo-controlled study was carried out with a St. John's wort extract (2.5-5:1, ethanol 60%; 5% hyperforin, no data on naphthodianthrones) in 72 patients with mild to moderate major depressive disorder in accordance with DSM-IV criteria. The daily dose was 3 × 300 mg of extract or placebo. Group differences in favour of the verum group were statistically significant after 4 weeks ($p = 0.011$) and 6 weeks ($p<0.001$). Average HAMD scores decreased from 19.7 to 8.9 in the St. John's wort group and from 20.1 to 14.4 in the placebo group. Analysis of responders showed a reduction of at least 50% from HAMD baseline scores in 62.2% of the St. John's wort group and 42.9% of the placebo group ($p = 0.10$). The difference was larger when '60%-responders' were considered (51.4% vs. 17.1%). Based on an adaptive interim analysis, the study was stopped after 6 weeks because convincing efficacy had already been demonstrated; however, according to an exponential regression model, the data suggested considerable potential for further HAMD reduction if treatment were continued with the St. John's wort extract, but not with placebo [36].

In a multicentre, randomized, double-blind, three-armed study, 263 primary care patients (mean age 47 years) with a diagnosis of moderate depression (ICD-10: F32.1 and F33.1) were allocated to 3 × 350 mg of a St. John's wort extract (5-7:1, 60% ethanol; 0.2-0.3% of total hypericin and 2-3% of hyperforin by HPLC) or 100 mg of imipramine (50 mg + 25 mg + 25 mg) or placebo daily for 8 weeks. The primary endpoint was the change from baseline in HAMD scores (mean initial score 22.6 points). The St. John's wort extract more effectively reduced HAMD scores than placebo (mean decrease after 6 weeks of 13.4 versus 10.3). Mean decreases in scores from baseline at 8 weeks were similar for St. John's wort extract and imipramine (15.4 versus 14.2). More patients receiving St. John's wort extract had ≥ 50% improvement in HAMD scores than did patients receiving placebo ($p = 0.027$); the proportions did not differ between St. John's wort and imipramine ($p = 0.14$). Comparable results were found for HAMA and CGI scores, and were most pronounced for the Zung self-rating depression (SDS) score. Quality of life as measured by the SF-36 standardized mental component scale was more improved by both active treatments than by placebo (40.5% more by St. John's wort extract, 30% more by imipramine). Using the SF-36 physical component scale, quality of life compared with placebo was markedly improved only by St. John's wort extract (74.5% more by St. John's wort extract, 23% by imipramine) [37].

The effects of 2 × 400 mg of a St. John's wort extract (5-7:1, ethanol 60%) or 2 ×10 mg of fluoxetine daily were compared in a 6-week randomized, double-blind, comparative trial (without a placebo group) involving 149 elderly outpatients (mean age 69 years) suffering from a first episode of mild or moderate depression (ICD-10: F32.0 or F32.1). Over the study period average HAMD scores decreased from 16.6 to 7.9 in the St. John's wort group and from 17.2 to 8.1 in the fluoxetine group. The efficacy of the two medications was found to be equivalent in both mild and moderate depressive episodes (no statistical confirmation of equivalence; no data on standard deviation or confidence intervals). Responder rates after 6 weeks (defined by a total HAMD score of not more than 10 or a reduction of at least 50% from initial score) were 71% in the St. John's wort group and 72% in the fluoxetine group [28].

In a drug surveillance study, 2404 patients (mean age 50 years) with depressive symptoms of varying severity were treated with a St. John's wort extract (5-7:1, 60% ethanol). The average dose was 120-180 mg of extract twice daily and the average treatment period 5 weeks. Typical symptoms of depression decreased in both frequency and intensity, the response rates (defined by the proportion of patients showing marked improvement or a symptom-free condition) with respect to individual symptoms being: depressed mood (73%), loss of interest (81%), reduced self-esteem and self-confidence (66%), lack of concentration (69%) and anxiety symptoms (68%). Overall, 90% of patients responded to treatment and more than 50% noticed an improvement in symptoms 2 weeks after commencement of therapy. Using the CGI scale, the investigators rated efficacy as good or very good in 77% of patients (about 80% for patients younger than 54 years, decreasing to 73% with older patients). A correlation was evident between severity of condition and response rates (82% for mild, over 79% for moderate, 64% for marked severity) [82].

In another drug monitoring study, 607 patients (mean age 50.5 years) with depressive symptoms were treated once or twice daily for 6 weeks with 425 mg of a St. John's wort extract (5-7:1; ethanol 60%). The initial mean HAMD score of 19.2 had dropped to 7.4 points by the end of the treatment. Similar results were

obtained using the von Zerssen self-rating depression scale, the score decreasing from 22.2 to 8.9 points. Core symptoms such as low mood, lack of energy and concentration improved by 50% [83].

*Clinical studies and drug surveillance studies in **mild to moderate depression** performed with hydroethanolic extracts (50% ethanol)*

In a randomized, double-blind, multicentre trial, 97 outpatients (mean age 43 years) with a moderate depressive episode (ICD-10, F32.1) were allocated to receive 2 × 100-120 mg of a St. John's wort extract (5-8:1; ethanol 50%) or placebo daily for 6 weeks. By the end of the study initial HAMD scores had dropped from 24.6 to 7.9 points in the St. John's wort group and from 22.6 to 10.3 in the placebo group, a statistically significant difference ($p = 0.019$). Responder rates (defined by a total score at the endpoint of less than 10 or a drop of at least 50% from initial score) were 79% and 56% respectively [42].

In a randomized, double-blind, multicentre trial with 162 outpatients (mean age 43 years) suffering from mild to moderate depression (ICD-10: F32.0, F32.1) the treatment was either 2 × 250 mg of a St. John's wort extract (4-7:1, 50% ethanol; 0.2% total hypericin) or placebo daily for 6 weeks. Over the course of the study HAMD scores dropped from 20.1 to 10.5 in the St. John's wort group and from 18.8 to 17.9 in the placebo group, a significant difference ($p<0.001$). Responder rates (defined by an endpoint score of not more than 10 or a drop of at least 50% from initial score) were 56% and 15% respectively [43].

In another randomized, double-blind, multicentre study, involving 240 subjects (mean age 47 years) with mild to moderate depression (ICD-10: F32.0, F32.1) and HAMD scores in the range 16-24 (mean initial score 19.6), the treatment was either 2 × 250 mg of a St. John's wort extract (4-7:1, 50% ethanol; 0.2% total hypericin) or 1 × 20 mg of fluoxetine daily for 6 weeks. No placebo group was included. After 6 weeks, HAMD scores had dropped from 19.65 to 11.5 in the St. John's wort group and from 19.5 to 12.2 in the fluoxetine group. Statistical analysis of the main outcome variable, the change in mean HAMD score, confirmed the equivalence of the two treatments with regard to overall antidepressant effect. In analysis of secondary variables, there was a trend in favour of St. John's wort in improving the absolute HAMD score ($p = 0.09$). Responder rates (defined by an endpoint score of not more than 10 or a drop of at least 50% from initial score) were 60% for the St. John's wort extract and 40% for fluoxetine ($p = 0.05$) [44].

In a randomized, double-blind, multicentre study with 324 outpatients (mean age 46 years) with mild to moderate depression (ICD-10: F32.0, F32.1, F33.0, F33.1), the treatment was either 2 × 250 mg of a St. John's wort extract (4-7:1, 50% ethanol; 0.2% total hypericins) or 2 × 75 mg of imipramine daily for 6 weeks. No placebo group was included. By the end of the study, HAMD scores had dropped from 22.4 to 12.0 in the St. John's wort group and from 22.1 to 12.75 in the imipramine group. Neither this difference nor differences between treatment groups in predefined secondary efficacy parameters were statistically significant, except for a difference in HAMD anxiety-somatisation subscale scores in favour of St. John's wort ($p = 0.03$). Responder rates, defined by a drop of at least 50% of the baseline HAMD values, were 43% in the St. John's wort group and 40% in the imipramine group [45].

In an open, multicentre drug surveillance study, 170 patients (mean age 49 years) with masked, mild, moderately severe or severe depression received 2 × 250 mg of a St. John's wort extract (4-7:1, 50% ethanol; 0.2% total hypericin) daily for an average treatment period of 66 days and were monitored with regard to efficacy and safety. HAMD scores were evaluated in 84 patients at the beginning and end of the study. Treatment response was satisfactory in patients with masked, mild and moderate depression but not in those with severe depression. Typical depressive symptoms, summarized in an unvalidated complaint score, decreased by 40% in the former group but by only 12.5% in the subgroup with severe depression. In the subgroup of patients whose HAMD scores were evaluated, the mean initial score of 36.3 dropped to 27.2 by the end of therapy. Again in a subgroup analysis, severe cases did not significantly benefit from the treatment (descriptive p-value of 0.46). Global efficacy, judged in 94 patients, was rated as good or very good in 78%, slight in 3% and insufficient in 19% of this subgroup [91].

Clinical studies performed with tinctures (49-50% ethanol)

In the first randomized, double-blind, placebo-controlled study of a St. John's wort preparation, 60 depressive patients (mean age 49 years) were treated daily for 6 weeks with 3 × 1.5 ml of a St. John's wort tincture (50% ethanol, daily dose equivalent to 0.9 mg of total hypericins) or placebo. The types of depression were classified as psychogenic, climacteric, somatogenic, involutional or juvenile. Using a self-developed, graded scale with 52 symptoms, the authors demonstrated a considerable reduction of 61.4% in the total score in the St. John's wort group compared to only 15.8% in the placebo group. Responder rates, defined by the investigators' assessment of good or very good improvement, were 63% in the St. John's wort group and 10% in the placebo group. Statistical parameters were not reported [24].

In another randomized, double-blind, placebo-controlled study, 49 patients (mean age 42.3 years)

with mild to moderate depressive symptoms were treated with 3 × 1 ml of a St. John's wort tincture (49% ethanol, 0.4:1) or placebo daily for 4 weeks. In the placebo group the mean number of symptoms had increased from 24 to 29.6 after 4 weeks, whereas patients treated with St. John's wort experienced an average reduction from 22.9 to 16.4 symptoms ($p<0.05$) [25].

In a two-centre, double-blind, placebo-controlled study, 40 patients (mean age 47 years) with depressive symptoms received daily either 3 × 1.5 ml of a St. John's wort tincture (49% ethanol, 0.4:1) or placebo. After 4 weeks, results from 16 patients in the St. John's wort group and 12 in the placebo group showed that initial HAMD total scores had decreased from 29.25 to 9.75 in the St. John's wort group and from 29.5 to 19.5 in the placebo group. Responder rates (defined by a drop of at least 50% from baseline HAMD scores or an endpoint score of 10 or less) were 62.5% in the St. John's wort group and 33.3% in the placebo group [32].

In a multicentre, double-blind, placebo-controlled study, 120 outpatients (mean age 48.5 years) suffering from mild depressive symptoms (ICD-09: 304.4 and 309.9; initial HAMD scores of 16-20) were treated with 3 × 1.5 ml of a St. John's wort tincture (49% ethanol; 0.25 mg of total hypericins per ml) or placebo daily for 6 weeks. Results from 116 patients were presented for quantitative analysis. In terms of overall symptomatology, there was a marked reduction of 57.9% in HAMD total scores in the St. John's wort group (from 21.6 to 8.9) compared to only 18.1% in the placebo group (from 20.9 to 16.1). Comparable results were obtained using the HAMA scale and von Zerssen's self-rating scale depression scale. Responder rates (defined by a total HAMD score of not more than 10 at endpoint or a drop of at least 50% from baseline scores) were 65.9% in the St. John's wort group and 25% in the placebo group [33].

In another randomized, double-blind, placebo-controlled, multicentre study, 88 outpatients (mean age 43.3 years) suffering from mild to moderate depressive symptoms (ICD-09: 300.4; HAMD score at least 16) were treated with either 3 × 1.5 ml of a St. John's wort tincture (49% ethanol; 0.25 mg of total hypericins per ml) or placebo daily for 4 weeks. HAMD scores dropped from 17.8 to 5.2 in the St. John's wort group ($p<0.001$) and from 17.3 to 15.5 in the placebo group. Responder rates, defined by a total HAMD score at endpoint of not more than 10 or a drop of at least 50% from baseline scores, were 70.7% in the St. John's wort group and only 7.1% in the placebo group [27].

In other studies, the same preparation at the same daily dosage was compared to 50 mg of imipramine daily in 30 patients with depressive states after surgery [53], and to 6 mg of bromazepam daily in 80 patients suffering from psychogenic depressive symptoms [26]. Comparable efficacy results for the St. John's wort tincture and the reference medication were reported in both studies.

In an open, comparative study in a gynaecological practice, patients (mean age 52.9 years) with climacteric complaints were treated daily with either 3 × 1.5 ml of a St. John's wort tincture (50% ethanol; daily dose equivalent to 0.9 mg of total hypericins; n = 40) or 3 × 2 mg of diazepam (n = 20). Treatment response was evaluated after 1 and 3 months using the CGI scale for overall efficacy, the HAMA scale and the Zung Self Rating Depression Scale (SDS). Subjective data on hot flushes, increased perspiration and general well-being were recorded in patient's diaries. In the investigator's judgement, 77.5% of patients treated with St. John's wort and 50% of patients treated with diazepam were fully remitted after 3 months. The score for the depression component of the HAMA scale (no HAMA total scores reported) dropped from 2.80 to 1.73 after 1 month and to 0.79 after 3 months in the St. John's wort group, and from 2.95 to 1.86 after 3 months in the diazepam group. Similar results were obtained from SDS score evaluation. No quantitative data from the patients' diaries were reported [52].

Clinical studies performed with other preparations

In a randomized, double-blind, multicentre, three-armed study involving 348 outpatients (mean age not reported) suffering from mild to moderate depression diagnosed in accordance with ICD-10, three preparations containing a dry extract (4-5:1) from fresh shoot tips of St. John's wort (standardized to 0.17 mg, 0.33 mg or 1 mg of total hypericins per day) were assessed for efficacy and safety over a treatment period of 6 weeks. The highest daily dose corresponded to 3 × 60 mg of the extract. Mainly for ethical reasons, no placebo group was included. In the per protocol analysis of the main outcome measure, initial average HAMD scores of 16-17 dropped to 8-9 after 6 weeks in all three groups. Response rates (defined by a decrease in the score to below 10 or a reduction of at least 50% from the initial score) were 62%, 65% and 68% respectively in the intention to treat analysis. No statistically significant differences between the three groups were detected [46].

In a randomized, double-blind, crossover study, the antidepressant effect of a St. John's wort tea taken twice daily (at least 0.28 mg of total hypericins per day) was assessed in 19 patients (at least 60 years old) and compared to milfoil tea (*Achillea millefolium*) as a control. Each treatment period lasted 14 days, separated by a wash-out period of 3 days. Thirteen patients reported better results with St. John's wort tea, while five patients had better results with milfoil

tea and one patient showed no difference. The pilot study indicated a trend towards a better mood in patients treated with St. John's wort tea ($p = 0.06$) [47].

Pharmacokinetic properties

Pharmacokinetics in animals

A study of the absorption and distribution of orally administered radioactively labelled ^{14}C-hypericin and ^{14}C-pseudohypericin in mice showed that 6 hours after administration 80% of hypericin and 60% of pseudohypericin had been absorbed. The distribution was not indicative of selective accumulation in certain organs. Most radioactivity was found in the blood, but radioactivity was also present in the brain [179].

The tissue uptake and distribution of hypericin was measured in rabbits and nude mice transplanted with P3 human squamous cell carcinoma. Maximum levels were attained 4 hours after intravenous administration to rabbits. The lungs had 5-fold higher levels than the spleen followed by liver, blood and kidney. Mice were investigated after acute administration and after 3 and 7 days of treatment. Peak concentrations were reached in murine organs after 4 hours. After 7 days of treatment elimination was rapid in most organs with a residue of < 10%, although 25-30% was retained in squamous cell tumours and in the brain, stomach and skin [180]. The tissue distribution of hypericin (2, 5 or 20 mg/kg, administered intraperitoneally) was studied in DBA/2 mice bearing subcutaneously implanted P388 lymphoma cells. Uptake was very high in the liver and spleen. Clearance of hypericin from plasma occurred at a fairly high rate and followed a two-phase exponential decay: a first phase of rapid clearance (half-life 6.9 hours) was followed by a slower phase (half-life 37.9 hours) [181].

After oral administration of an ethanolic extract containing 5% hyperforin to rats at 300 mg/kg, maximum plasma levels of 370 ng/ml hyperforin were reached after 3 hours. Estimated half-life and clearance values were 6 hours and 70 ml/min/kg [182].

Pharmacokinetics in humans

A study of the bioavailability of hypericin in 2 healthy volunteers after oral administration of a St. John's wort extract (300, 600 and 1200 mg at intervals of 7 days) demonstrated that plasma levels of hypericin were dose-dependent. After ingestion of a single dose of 600 mg of the extract by 12 volunteers, the following parameters were determined for hypericin: t_{max} 2.5 hours, c_{max} 4.3 ng/ml and a plasma half-life of about 6 hours [183].

Oral administration to 12 healthy volunteers (at intervals of at least 10 days) of single doses of a methanolic extract of St. John's wort containing 250, 750 and 1,500 µg of hypericin and 526, 1,578 and 3,135 µg of pseudohypericin respectively gave peak plasma levels of 1.3, 7.2 and 16.6 µg/litre for hypericin and 3.3, 12.2 and 29.7 µg/litre for pseudohypericin. C_{max} and AUC values for the lowest dose were disproportionately lower than those for the higher doses. Lag times were determined as 1.9 hours for hypericin and 0.4 hours for pseudohypericin. Mean half-lives for absorption, distribution and elimination were 0.6, 6.0 and 43.1 hours after 750 µg of hypericin, and 1.3, 1.4 and 24.8 hours after 1,578 µg of pseudohypericin. After 14 days of oral treatment with 250 µg of hypericin and 526 µg of pseudohypericin, steady state levels of 7.9 µg/litre for hypericin and 4.8 µg/litre for pseudohypericin were achieved. Kinetic parameters in two subjects after intravenous administration resembled those after oral administration. Hypericin and pseudohypericin were initially distributed into volumes of 4.2 and 5.0 litres respectively; at steady state the mean distribution volumes were 19.7 litres for hypericin and 39.3 litres for pseudohypericin; systemic bioavailability from the methanolic extract was about 14 and 21% respectively [184].

A methanolic St. John's wort extract was administered orally as single doses of 900, 1,800 and 3,600 mg, containing 2.81, 5.62 and 11.25 mg of total hypericins. Maximum plasma concentrations of total hypericin, observed about 4 hours after administration, were 0.028, 0.061 and 0.159 mg/litre. Phototoxic reactions could not be excluded for hypericin doses above 11.25 mg of total hypericin and plasma levels above 100 µg/litre [97].

Hypericin levels in serum and skin blister fluid were determined in volunteers after oral administration of a hydromethanolic extract as a single dose of 6 tablets or 3 × 1 tablet daily for 7 days. Each tablet contained 300 mg of St. John's wort extract, standardized to 900 µg of total hypericins. Six hours after the single high dose, mean levels of total hypericin were 43 ng/ml in serum and 5.3 ng/ml in skin blister fluid. After 3 tablets daily for one week, mean levels were 12.5 ng/ml in serum and 2.8 ng/ml in skin blister fluid. Hypericin levels in skin of >100 ng/ml are considered to be phototoxic [185].

Plasma levels of hyperforin were measured over a 24-hour period in volunteers treated with 300 mg of an ethanolic extract of St. John's wort containing 14.8 mg of hyperforin. Maximum plasma levels of about 150 ng/ml were reached 3.5 hours after oral administration. Half-life and mean residence time of hyperforin were 9 and 12 hours respectively. Up to 600 mg of the extract, hyperforin kinetics were linear [182].

Preclinical safety data

Systematic studies on single dose toxicity, reproductive toxicity and carcinogenicity of St. John's wort extracts

have been carried out by major manufacturers, but not published.

In vitro experiments

Hamster oocytes were incubated in St. John's wort extract before sperm/oocyte interaction. Penetration was prevented by a very high dose of 0.6 mg/ml, but 0.06 mg/ml had no effect [186]. Apparently this can be easily explained by the tannin content of the drug and does not indicate an antifertility effect.

Foetal calf serum or albumin strongly inhibited the photocytotoxic effects of pseudohypericin, but not those of hypericin, in A431 tumour cells. The authors concluded that hypericin is likely to be the constituent responsible for hypericism [187]. Human keratinocytes were cultured in the presence of different concentrations of St. John's wort extract and irradiated with UV-A or UV-B. A phototoxic effect was seen only on irradiation with UV-A at high hypericin concentrations (≥ 50 µg/ml) [188].

In vivo experiments

Effects on offspring

20 female mice were treated orally with 180 mg/kg/day of St. John's wort extract containing 0.3% of hypericin for 2 weeks before conception and throughout gestation. Perinatal outcomes, growth and physical milestones of the offspring were compared to a control group. Gestational ages at delivery and litter sizes did not differ between the groups. Body weight, body length and head circumference from postnatal day 3 to adulthood did not differ regardless of gender. The only difference between the groups was a temporary delay in the eruption of upper incisors in male offspring exposed to St. John's wort. Reproductive capacity, perinatal outcomes and growth and development of second-generation offspring were unaffected by treatment with St. John's wort extract [189].

Human studies

An experimental, double-blind, placebo-controlled study with 40 volunteers showed that photosensitivity was not induced by therapeutically relevant dosages of total hypericin, i.e. up to 1 mg daily for 8 days [190]. In a study involving intravenous administration of synthetic hypericin to HIV-infected patients, (reversible) symptoms of phototoxicity were observed at the highest dosage regime, which was 35 times higher than the highest oral dosage of total hypericin used in the therapy of depressive disorders [191].

Following administration of a methanolic extract to human volunteers as single doses of 900, 1800 and 3600 mg (containing 2.81, 5.62 and 11.25 mg of total hypericins respectively) and subsequent irradiation with solar-simulated irradiation or UV-A only, sensitivity to UV-A light increased slightly at the highest dose, but no correlation was found between plasma total hypericins levels and photosensitivity. After 15 days of treatment with 3 × 600 mg of the extract (5.6 mg total hypericins/day) sensitivity to both forms of irradiation had increased significantly; this effect could be compensated by reducing irradiation time by 21% [97].

Hypericin levels in skin of more than 100 ng/ml are considered to be phototoxic [184].

Photosensitization caused by St. John's wort has mainly been reported in veterinary studies, especially in unpigmented skin of grazing animals [192,193]. Dose-dependent phototoxic symptoms were observed in calves within 4 hours after single oral doses of dried St. John's wort at 3-5 g/kg body weight [194]. Merino ewes (an unpigmented breed, freshly shorn of wool), dosed orally with 5.7, 4.0 or 2.85 g/kg of dried St. John's wort (corresponding to 5.3, 3.7 or 2.65 mg/kg of hypericin) and then exposed to bright sunlight, had a tolerance level for hypericin of less than 2.65 mg/kg [192].

Clinical safety data

St. John's wort extracts have a particularly high level of clinical safety. Preparations have been studied in more than 13,900 patients, in whom good tolerability has generally been proven.

In 17 randomized, placebo-controlled clinical studies of St. John's wort, the few adverse effects noted were mainly headache, dizziness, sleep disorders, itching or non-specific gastrointestinal disturbances [24,25, 27,29-36,38,42,43,50,57,92].

In comparative clinical studies against synthetic antidepressants, the incidence of adverse events reported for St. John's wort preparations is generally higher than in placebo-controlled studies; this is considered a psychodynamic phenomenon resulting from the informed consent instructions in the double-blind design, in which the clinical investigator has to mention all possible side effects which might occur with both treatments offered. However, the overall rate of side-effects is still more favourable for St. John's wort than for synthetic antidepressants [60]. Pooled data from 8 studies representing more than 1,400 patients show that the proportions of patients reporting "any" side effects were 23.9% with St. John's wort preparations compared to 40.5% with standard antidepressants [28,39-41,44,45,56,93].

Two three-armed studies have compared a St. John's wort extract with both a synthetic antidepressant and placebo. In the first study, the rate of adverse events was 46% in an imipramine group, 22% in the St. John's wort group and 19% in the placebo group [37]. In the second study, the absolute rates of adverse events were not stated but, from the adverse events that

differed significantly by treatment, the average rates for the events were 25% in a sertraline group (with diarrhoea occurring in 38% of the patients), 22% in the St. John's wort group (with frequent urination occurring in 27%) and 15% in the placebo group (with forgetfulness in 22%) [57].

In more naturalistic settings such as drug monitoring studies, subjective adverse events may be assessed in a more suitable quantitative and qualitative way. From these studies, the incidence of total adverse events among treated patients was 1-3% [81-91]. For comparison, the rate of adverse events in observational studies with tricyclic antidepressants is between 30 and 60% and with selective serotonin reuptake inhibitors (SSRIs) between 15 and 30% [60]. The most frequent side effects in the two largest St. John's wort studies, covering a total of 5,654 patients [82,85], were reported as mild gastrointestinal symptoms (0.42% [82], 0.55% [85]) such as stomach-ache, nausea, diarrhoea or constipation; allergic reactions such as pruritus and exanthema (0.52% [85]); fatigue (0.4% [85]); anxiety and restlessness (0.21% [82], 0.26% [85]); dizziness (0.12% [82], 0.15% [85]) and headache (0.12% [82]). According to an official Adverse Drug Reactions recording system, reversible skin reactions (photosensitization) have been reported in 1 per 300,000 cases treated with St. John's wort preparations [60], which is very rare. Significant phototoxicity occurred only in HIV-infected persons after administration of intravenous hypericin, 0.25-0.5 mg/kg twice weekly or 0.25 mg three times daily, or oral hypericin 0.25 mg/kg daily [98].

Systematic studies evaluating long-term side effects of St. John's wort are not available.

A detailed overview of the pharmacological, toxicological and clinical literature on St. John's Wort is given in two recent reviews [7,195].

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JUNIPERI PSEUDO-FRUCTUS

Juniper

DEFINITION

Juniper consists of the dried ripe cone berry of *Juniperus communis* L. It contains not less than 10 ml/kg of essential oil, calculated with reference to the anhydrous drug.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

Essential oil (up to 3.0% V/m) [2] of very variable composition depending on the source but consisting mainly of monoterpene hydrocarbons, principally α-pinene. Monoterpene alcohols including terpinen-4-ol and sequiterpenes such as β-caryophyllene are also present [2-6]. About 105 constituents occur in the oil [2]. Analysis of essential oil from 15 batches of juniper from various sources gave results in the ranges: α-pinene (24.1-55.4%), β-pinene (2.1-6.0%), myrcene (7.3-22.0%), sabinene (1.4-28.8%), limonene (2.3-10.9%), terpinen-4-ol (0.7-17.0%), α-terpineol (up to 1.7%), terpinolene (0.7-1.9%), γ-terpinene (0.5-5.8%), α-terpinene (0.5-2.6%), α-thujene (0.6-1.9%) and caryophyllene (1.3-2.3%) [5].

Other constituents include condensed tannins [7], flavonoids [8,9], diterpene acids, aldehydes and alcohols [10,11], fatty alcohols [11] and about 30% of glucose and fructose [12].

CLINICAL PARTICULARS

Therapeutic indications

Juniper has widely documented uses as a remedy to enhance the renal elimination of water [12-15] and for dyspeptic complaints [12-14]. Published scientific evidence does not yet adequately support these therapeutic indications.

Posology and method of administration

Dosage

Adults: 2-3 g of dried berries as an infusion in 150 ml of hot water, 3-4 times daily [13]. Tincture (1:5 in ethanol 45%), 1-2 ml three times daily [14].

Method of administration

For oral administration.

E/S/C/O/P
MONOGRAPHS
Second Edition

Duration of administration
Juniper should not be used for more than 4 weeks without consulting a doctor [13].

Contra-indications
Acute or chronic inflammation of the kidney [13,16].

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
Juniper may influence glucose levels in diabetics [17].

Pregnancy and lactation
Should not be used during pregnancy and lactation [18,19]. Abortifacient activity of juniper has been observed in rats after oral administration of a 50% ethanolic extract at 300 mg/kg bodyweight [19].

Effects on ability to drive and use machines
None known.

Undesirable effects
None for juniper.

In the past, based on very old reports (1937 and earlier, but still reiterated in secondary literature), adverse effects such as kidney irritation have been associated with juniper and juniper oil. However, a comprehensive review of the literature concluded that such reports are unreliable; they related only to juniper oil and adverse effects were probably due to contamination with turpentine oil [16,20].

Overdose
No toxic effects reported for juniper.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vivo experiments

Diuretic effects
A 10% aqueous infusion of juniper or a 0.1% aqueous solution of juniper oil (with 0.2% of Tween 20 solubilizer) or a 0.01% aqueous solution of terpinen-4-ol were orally administered to groups of rats at 5 ml/100 g body weight; control groups were given water or water + 0.2 % Tween orally and reference groups were given antidiuretic hormone (ADH; vasopressin) intraperitoneally at 0.004, 0.04 or 0.4 IU/100 g. Compared to water, the 10% aqueous infusion of juniper and the 0.1% aqueous solution of juniper oil (in which the ratio of pinene fraction to terpinen-4-ol was 5:1) caused reductions of only 6% in diuresis over a 24-hour period, equivalent to the effect of 0.004 IU/100 g of ADH, while the 0.01% solution of terpinen-4-ol caused a reduction of 30% in diuresis ($p<0.01$), equivalent to 0.4 IU/100 g i.p. of ADH. However, after continued daily administration at the same dose levels, the two juniper preparations and terpinen-4-ol stimulated diuresis on days 2 and 3, although only the 10% aqueous infusion of juniper exerted significant diuretic activity (+ 43% on day 2; + 44% on day 3; $p<0.05$), suggesting that the diuretic effect is due partly to the essential oil and partly to hydrophilic constituents [21].

After oral administration to rats of a lyophilised aqueous extract of juniper at 1000 mg/kg body weight, no increase in urine volume or excretion of Na^+, K^+ or Cl^- ions could be demonstrated over a 6-hour period compared to the effect of the same volume of water [22].

Oral administration to rats of an aqueous infusion equivalent to 125 mg of juniper increased urine amount by 36% and chloride excretion by 119% compared to animals given water only [23]. In similar experiments, urine volume increased by 20% in rabbits after the equivalent of 750 mg of juniper and by 38% in mice after the equivalent of 50 mg of juniper [24]. However, in all cases, twice or half these doses had much less or no effect [23,24].

No significant diuresis was observed in rats after oral administration of juniper oil at 100 or 333 mg/kg/day for 28 days [25].

Subcutaneous injection of juniper oil (1 ml/kg) into rats produced significant levels of diuresis after 4 and 24 hours compared to a control (sodium chloride solution). Terpinen-4-ol isolated from the oil and injected subcutaneously at a dose of 0.1 ml/kg showed almost twice the diuretic activity of the oil [26]; increased amounts of K^+, Na^+ and Cl^- were also excreted [27].

Anti-inflammatory effects
A dry 80%-ethanolic extract of juniper, administered orally at 100 mg/kg, reduced carrageenan-induced rat paw oedema by 60% ($p<0.001$) compared to 45% for indometacin at 5 mg/kg ($p<0.01$) [28].

Hypoglycemic effects
An orally administered decoction of juniper showed significant hypoglycemic activity in normal rats after single doses equivalent to 250-500 mg juniper/kg and in streptozotocin-induced diabetic rats after 24-day treatment with doses equivalent to 125 mg juniper/kg. The effects were attributed to an increase in peripheral absorption of glucose, independent of plasma insulin levels [17]. However, a subsequent study failed to show an antihyperglycaemic effect of juniper in streptozotocin-induced diabetic mice [29].

Other effects
Intravenous administration of a lyophilised aqueous extract of juniper (25 mg/kg body weight) to normotensive rats produced an initial transient rise in arterial pressure followed by a reduction of 27%. A dose of 1.2 g/kg of the same extract produced an analgesic response of 178% as measured by thermal stimuli in mice [22].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
The intraperitoneal LD_{50} of a lyophilized aqueous extract of juniper was calculated as 3 g/kg body weight in mice [22]. No mortality occurred and no side effects were apparent in rats after a single oral dose of 2.5 g/kg body weight of a dry 80%-ethanolic extract of juniper [28].

Juniper oil had an oral LD_{50} of 6.28 g/kg in the rat [30]. The acute dermal LD_{50} of the oil exceeded 5 g/kg in rabbits [31].

The LD_{50} of terpinen-4-ol was 0.75 ml/kg in mice after subcutaneous injection and 0.78 ml/kg after intramuscular injection; in rats, the LD_{50} after intramuscular injection was 1.5 ml/kg [27].

Chronic toxicity
Chronic administration of terpinen-4-ol at therapeutic dose levels caused no pathological changes in the rat [27].

Nephrotoxicity
The oral toxicity, especially possible nephrotoxicity, of two juniper oils of good pharmaceutical quality were tested in rats for 28 days in two series of experiments. In the first series, rats were treated with 100, 333 or 1000 mg of oil/kg body weight/day with an α-pinene + β-pinene to terpinen-4-ol ratio of 3:1. In the second series, rats received 100, 300 or 900 mg of oil/kg/day with an α-pinene + β-pinene to terpinen-4-ol ratio of 5:1; an additional group received 400 mg/kg/day of terpinen-4-ol, a constituent of the oil with postulated diuretic effects. From biochemical, pathological and histological investigations, neither of the juniper oils nor terpinen-4-ol produced nephrotoxic effects and they were considered non-toxic at therapeutic dose levels [25].

Juniper oils with a relatively low content of pinenes (monoterpene hydrocarbons less than 60%) and a high terpinen-4-ol content have been recommended for pharmaceutical use [16,20].

Teratogenicity
No evidence of teratogenicity was observed in rats after oral administration of a dry 50% ethanolic extract of juniper at 300-500 mg/kg bodyweight [19].

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LICHEN ISLANDICUS

Iceland Moss

DEFINITION

Iceland moss consists of the whole or cut dried thallus of *Cetraria islandica* (L.) Acharius s.l.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

Polysaccharides (over 50%), principally lichenan (or lichenin), a hot water-soluble, linear β-D-glucan with 1→4 and 1→3 links (ratio greater than 2:1) [2-4] and isolichenan (or isolichenin), a cold water-soluble, linear α-D-glucan with 1→3 and 1→4 links (ratio approx. 55:45) [5,6]. An α-D-glucan (denoted as Ci-3) resembling isolichenan but with a much higher degree of polymerization [7], a branched galactomannan [8,9] and an acidic, branched polysaccharide containing D-glucose and D-glucuronic acid units [10] are also present.

Other characteristic constituents are bitter-tasting lichen acids including the depsidones fumarprotocetraric acid (2.6-11.5%) [11,12] and protocetraric acid (0.2-0.3%) [12], and the aliphatic lactone protolichesterinic acid (0.1-0.5%) [11].

CLINICAL PARTICULARS

Therapeutic indications

Dry cough; irritation or inflammation of the oral and pharyngeal mucosa [13-18].

Iceland moss is also widely documented as a bitter remedy for lack of appetite [15-18].

Posology and method of administration

Dosage

For upper respiratory tract ailments

Adult daily dose: 3-8 g of the drug as a decoction or equivalent liquid preparation [15-20], taken in small amounts as required. In the form of pastilles containing aqueous extract from 50-300 mg of the drug, 10 or more daily [13].

As a bitter

Adult single dose: 1-2 g of the drug [15] as a cold macerate, infusion, tincture or other bitter-tasting preparation [15-18, 21].

Elderly: dose as for adults.

Children, average daily dose: 1-4 years of age, 1-2 g; *4-10 years,* 2-4 g; *10-16 years,* 4-6 g [22].

Method of administration
For oral administration in liquid or solid dosage forms. In the treatment of respiratory tract ailments, the addition of sweetener to liquid preparations is recommended to mask the bitter taste [17]; pastilles should be sucked slowly in the mouth [14].

Duration of administration
No restriction.
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Immunomodulatory effects
Immunostimulating activity, demonstrated by enhancement of phagocytosis using human granulocytes, was exhibited by two polysaccharides isolated from Iceland moss, an alkali-soluble galactomannan denoted as KI-M-7 [9] and a water-soluble neutral α-D-glucan denoted as Ci-3 [7], as well as by a hot water extract of Iceland moss and polysaccharide fractions from it [23].

Anti-inflammatory activity
A hot water extract of Iceland moss, and also the α-D-glucan Ci-3, showed activity in a haemolytic anti-complementary assay [7].

The activity of 5-lipoxygenase in porcine leucocytes was inhibited by protolichesterinic acid with an IC_{50} of 20 μM [24]. Leukotriene B_4 biosynthesis in stimulated bovine polymorphonuclear leukocytes was inhibited by protolichesterinic acid with an IC_{50} of 9 μM ($p<0.05$) [25].

Antiproliferative activity
Antiproliferative and cytotoxic effects exhibited by protolichesterinic acid may be related to its inhibitory activity on 5-lipoxygenase. At an ED_{50} between 1.1 and 24.6 μg/ml protolichesterinic acid caused a significant reduction in DNA synthesis in three malignant human cell lines (T-47D and ZR-75-1 from breast carcinomas and K-562 from erythroleukaemia); significant cell death occurred in all three cell lines at concentrations above 20 μg/ml. The proliferative response of mitogen-stimulated peripheral blood lymphocytes was also inhibited with a mean ED_{50} of 8.4 μg/ml. In contrast, DNA synthesis, proliferation and survival of normal skin fibroblasts were not affected at doses of up to 20 μg/ml [26].

Antibacterial activity
Inhibitory activity of protolichesterinic acid against 35 strains of *Helicobacter pylori* has been demonstrated. The MIC_{90} was 32 μg/ml, considerably higher than that of ampicillin (0.125 μg/ml) and erythromycin (0.25 μg/ml) but only twice as high as that of metronidazole (16 μg/ml) [27].

In a study of the activity of protolichesterinic acid against *Mycobacterium aurium,* a non-pathogenic organism with a similar sensitivity profile to *M. tuberculosis,* the MIC was found to be too high (250 μg/ml compared to 0.25 μg/ml for streptomycin) to merit further investigation of antimycobacterial potential [28].

Antiviral activity
Protolichesterinic acid has been shown to be a potent inhibitor of human immunodeficiency virus-1 reverse transcriptase with an IC_{50} of 24 μM [29].

Other effects
In a study of the bioadhesive effects of purified (> 95%) polysaccharides from medicinal plants on porcine buccal membranes, polysaccharides from Iceland moss showed only slight adhesion to epithelial tissue whereas moderate adhesion was observed with polysaccharides from *Althaea officinalis* and *Plantago lanceolata* and strong adhesion with polysaccharides from *Fucus vesiculosus* and *Calendula officinalis* [30].

In vivo experiments

Immunomodulatory effects
A hot water extract at 1 mg/kg body weight [23] and

an alkali-soluble galactomannan (isolated from Iceland moss) at 10 mg/kg [9], administered intraperitoneally, exhibited marked immunomodulating activity in the carbon clearance assay in mice, substantially increasing the rate of reticuloendothelial phagocytosis. Both substances stimulated the rate of removal of injected colloidal carbon particles from the bloodstream by a mean ratio of 1.9 compared to controls.

Cognitive effects

Synaptic plasticity in the hippocampal area of the brain is important in the initial storage of certain forms of memory. Isolichenan isolated from Iceland moss and administered intravenously to rats at 1 mg/kg body weight significantly enhanced short-term potentiation of hippocampal synaptic plasticity ($p<0.05$), evoked by high-frequency sub-threshold tetanic stimulation (20 pulses at 60 Hz) as an approximation to learning stimulus [31,32].

When isolichenan was orally administered to rats at 100 mg/kg it significantly repaired the effect of β-amyloid peptide-induced memory impairment ($p<0.05$) in the Morris water maze test, which depends heavily on intact hippocampal function. Similarly, in mice with learning ability impaired by pretreatment with 30% ethanol, oral isolichenan significantly improved memory acquisition in passive-avoidance tests ($p<0.01$ at 100 mg in step-through tests, $p<0.01$ at 400 mg/kg in step-down tests). No effect was observed on the cognitive performance of healthy rats or mice [31,32].

Clinical studies

In a comparative double-blind study, 63 patients with inflammation and dryness of the oral cavity due to breathing only through the mouth after nasal surgery were divided into three random groups and treated daily with 10 pastilles, each containing aqueous extract equivalent to: 0.048 g (n = 23) or 0.3 g (n = 18) or 0.5 g (n = 22) of Iceland moss. Treatment commenced on the day after surgery and continued for 5 days. Assessments by physicians, using biometric observations on a 0-3 scale, of coating, dryness and inflammation of the mucosa, conspicuousness of lymph nodes, tongue coating and symptoms such as hoarseness and sore throat revealed a similar and substantial degree of improvement in all three groups over the 5-day treatment, indicating that the lowest dosage, equivalent to 10 × 0.048 g (approximately 0.5 g) of Iceland moss daily, was sufficient [13].

In an open study, 100 patients aged between 7 and 85 years with pharyngitis, laryngitis or acute/chronic bronchial ailments were treated with pastilles (1-2 pastilles every 2-3 hours), each containing aqueous extract from 160 mg of Iceland moss, for between 4 days and 3 weeks. The results were assessed as positive in 86 cases [14].

Pharmacokinetic properties

No data available

Preclinical safety data

Protolichesterinic acid showed no appreciable cytotoxic activity in *in vitro* tests with a variety of cultured mammalian cells [29].

Clinical safety data

In two clinical studies, involving the treatment of a total of 163 patients for 4-5 days (in some cases 3 weeks) with pastilles containing Iceland moss aqueous extracts in amounts corresponding to 0.5-5 g of crude drug daily, the preparations were well tolerated with an absence of side effects [13,14].

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LINI SEMEN

Linseed

DEFINITION

Linseed consists of the dried ripe seeds of *Linum usitatissimum* L.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

The seeds contain 3-9% of mucilage polysaccharides composed mainly of galacturonic acid, xylose, galactose and rhamnose units [2-4,5]; 30-45% of fixed oil [4] mainly consisting of triglycerides of linolenic (40-60% [6]), linoleic and oleic acids [2,7,8]; approx. 25% of protein [2,3]; and 0.1-1.5% of cyanogenic glycosides such as the diglucosides linustatin and neolinustatin (the glucosides of linamarin and lotaustralin respectively) [2-4,9,10]. Other constituents include secoisolariciresinol diglucoside, a precursor of lignans in mammals [11-14], and a serine proteinase inhibitor (LUTI = *Linum usitatissimum* trypsin inhibitor) [15].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Constipation [2-4,16-21].
Irritable bowel syndrome [17,18].
Diverticular disease [17,18].
Symptomatic short-term treatment of gastritis and enteritis [2-4,16,19,22].

External use

Painful skin inflammations [4,19,23].

Posology and method of administration

Dosage and method of administration

Adults and children over 12 years of age

Internal use

As a laxative: 5 g of whole, finely-cracked or freshly crushed seeds, soaked in water and taken with a glassful of liquid three times daily. The effect starts 18-24 hours later [3,4,16,19,24].

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As a demulcent for gastritis and/or enteritis: for a mucilaginous preparation soak 5-10 g of whole linseed in 150 ml water and strain after 20-30 minutes [2,4,19].

External use
30-50 g of crushed or powdered seed (may be defatted) as a warm poultice or warm compress [4,19].

Children from 6 to 12 years of age: half the adult dose [25].
Children under 6 years of age: to be treated under medical supervision only.

Duration of administration
Because of the gradual mode of action of bulk-forming laxatives, treatment should be continued for a minimum of 2-3 days to ensure optimum benefit [26].

If abdominal pain occurs, or if there is no response after 48 hours, use of linseed should be discontinued and medical advice must be sought.

Contra-indications
Atonic and obstructive ileus, subileus or conditions likely to lead to intestinal obstruction. Acute abdominal pain of any origin (e.g. appendicitis) [2,18].

Special warnings and special precautions for use
Linseed (whole, finely-cracked or freshly crushed) should be soaked and taken with at least 10 times the amount of fluid, otherwise bezoar formation and intestinal obstruction may occur [27,28].

Persons with weight problems should take linseed whole, not cracked, because of its rich energy content of about 470 kcal (1960 kJ)/100 g [2].

Interaction with other medicaments and other forms of interaction
The absorption of other medications taken at the same time may be delayed [15,29].
Diabetics should be aware of a potential delay in glucose absorption [29-33].

Pregnancy and lactation
There are no reports of any harmful or deleterious effects during pregnancy and lactation [34].

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
In spite of its content of cyanogenic glycosides, single doses of up to 150-300 g of powdered linseed are not toxic. Health risks are not to be expected [2-4,6,16,19,35-38].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Laxative effect
Dietary fibre such as linseed binds with water and swells to form a demulcent gel in the intestine. As water bound to the fibre is prevented from being absorbed in the colon, the faeces are softened and the volume of bowel contents increases [2,4,16-18].

A decrease in transit time and increase of stool weight by physical stimulation of intestinal peristalsis have been demonstrated in two multicentric studies (n = 108 and n = 114) in patients suffering from constipation [20,21].

Effect on gastro-intestinal complaints
The mucilage has been reported to have a palliative effect in patients with pain associated with gastro-intestinal problems [2,4,16].

Clinical studies
In an open pilot study 70 patients suffering from various functional upper abdominal complaints such as sensations of pressure and repletion, loss of appetite, nausea, vomiting and heartburn were treated with an aqueous linseed mucilage preparation (1:10) at a dosage of 8 × 25 g (including a small amount of excipients) per day. All except three patients experienced improvements. After 3 days the total symptom score had significantly decreased ($p<0.01$). Each individual symptom score decreased on average, the largest reductions being observed for the sensation of pressure (41.5%) and the sensation of repletion (36.8%). In global assessments by both patients and physicians the efficacy was rated as good or very good in most cases [22].

Effect on blood glucose levels

Pharmacological studies in humans
Viscous types of dietary fibre may cause a delay in gastric emptying as shown in two studies with 11 and 7 healthy volunteers [29,30]. In these studies [29,30], and in one involving 8 non-insulin-dependent diabetic volunteers [31], it could be demonstrated that addition of certain types of dietary fibre to the diet significantly decreased postprandial hyperglycaemia. Thus an improvement in the control of blood-glucose concentration might be expected [31].

Effect on blood lipid levels

In vivo experiments
The effect of secoisolariciresinol diglucoside (SDG) was investigated in rabbits receiving either their normal diet (chow pellets, control group, n = 8), a control diet supplemented with SDG (15 mg/kg body weight/day, n = 5), a diet containing cholesterol (1%, n = 6) or

cholesterol + SDG (1% cholesterol + SDG 15 mg/kg body weight/day, n = 5). Blood samples were collected before the experiment, and after 4 and 8 weeks, then the aorta was removed for assessment of atherosclerotic plaques, malondialdehyde (an aortic tissue lipid peroxidation product) and aortic tissue chemiluminescence (a marker for antioxidant reserve). Serum total cholesterol (TC), LDL-cholesterol (LDL-C) and the ratio LDL-C/HDL-C and TC/HDL-C increased in the 3rd and 4th group. SDG significantly reduced TC and LDL-C by 33 and 35% respectively at week 8 ($p<0.05$), and significantly increased HDL-C by >140% at week 4 ($p<0.05$). It also decreased TC/HDL-C and LDL-C/HDL-C ratios significantly by approx. 64% ($p<0.05$). Comparing atherosclerotic plaques in the 3rd and 4th groups, it was found that SDG significantly reduced hypercholesterolaemic atherosclerosis by 73% ($p<0.05$). There were increases in aortic malondialdehyde and chemiluminescence in the 3rd group, but they were significantly lower in the 4th than in the 3rd group ($p<0.05$) [39].

Clinical studies

In a randomized, cross-over trial, 22 men and 7 postmenopausal women with hyperlipidaemia who followed a National Cholesterol Education Program (NCEP) Step II diet received fibre-rich muffins containing either partially defatted linseed (approx. 50g daily) or wheat bran as a control, both corresponding to approx. 20 g of fibre daily; for 3 weeks, the treatment phases being separated by at least 2 weeks. Linseed supplementation significantly reduced total cholesterol by 4.6% ($p = 0.001$), LDL cholesterol by 7.6% ($p<0.001$), apolipoprotein B by 5.4% ($p = 0.001$) and apolipoprotein A-I by 5.8% ($p = 0.070$) compared to the control, but had no effect on serum lipoprotein ratios. No significant effects were observed on serum HDL cholesterol, serum protein carbonyl content or *ex vivo* androgen and progestin activity [40].

Oestrogenic effects

In vitro experiments

Lignan precursors present in linseed are converted by bacteria present in the colon to metabolites interfering with metabolism and activity of oestrogens [13].

In vivo experiments

Experiments in pigs demonstrated the capacity of various fibres to bind to oestrogens [41]. It has therefore been suggested that linseed may lower the risk of oestrogen dependent tumours, e.g. some colon and mammary carcinomas [11-13,42].

Pharmacological studies in humans

Quantitative urine assays in 62 women studied 4 times during one year showed a significant positive correlation between the intake of fibre and urinary excretion of lignans and phytoestrogens and the concentration of plasma SHBG [42].

In an open randomized cross-over study involving 18 women with normal cycles, the effects of ingestion of linseed powder on the menstrual cycle was investigated. Each of them consumed her usual omnivorous, low fibre (control) diet for 3 cycles and her usual diet supplemented with linseed (10 g/day) for another 3 cycles. The second and third linseed cycles were compared to the corresponding control cycles. During these 36 control cycles, 3 anovulatory cycles occurred, compared to none during the 36 linseed cycles. Compared to ovulatory control cycles, the ovulatory linseed cycles were consistently associated with longer luteal phase (LP) lengths (mean 12.6 vs. 11.4 days; $p = 0.002$). There were no significant differences between linseed and control cycles in concentrations of oestradiol or oestrone during the early follicular phase, midfollicular phase or LP. Although linseed ingestion had no significant effect on LP progesterone concentrations, LP progesterone/oestradiol ratios were significantly higher during the linseed cycles. Midfollicular phase testosterone concentrations were slightly higher during the linseed cycles. Linseed ingestion had no effect on early follicular phase concentrations of DHEA-S, PRL or sex hormone-binding globulin [43].

Effects on β-glucuronidase activity

In vivo experiments

Six groups of Sprague-Dawley rats were fed one of the following diets for 100 days: a basal high-fat diet (20% fat), the basal diet supplemented with 2.5 or 5% of linseed, the basal diet supplemented with 2.5 or 5% of defatted linseed, or the basal diet with a daily dose of 1.5 mg of secoisolariciresinol diglucoside isolated from linseed. All rats were injected with a single dose of azoxymethane (15 mg/kg body weight) one week before treatment. Urinary lignan excretion, an indicator of mammalian lignan production, significantly increased in both the linseed groups and in the low-dose defatted linseed group ($p<0.0003$, $p<0.0001$ and $p<0.0001$ respectively) compared to the control. The total activity of caecal β-glucuronidase significantly increased in a dose-dependent manner in both the defatted linseed groups and in the high-dose linseed group ($p<0.01$, $p<0.047$ and $p<0.0004$ respectively). Compared to control the number of aberrant crypts per focus was significantly reduced ($p<0.01$ to $p<0.04$) in the distal colon of the five groups of treated rats. Four microadenomas and two polyps were observed in the control group but none in the treated groups. The total activity of β-glucuronidase was positively correlated with total urinary lignan excretion ($r = -0.280$, $p<0.036$, $n = 60$), and negatively with the total number of aberrant crypts ($r = -0.330$, $p<0.010$, $n = 57$) and the total number of aberrant crypt foci ($r = -0.310$, $p<0.018$, $n = 57$) in the distal colon. There were no significant differences between linseed and corresponding defatted linseed groups. It was concluded that linseed

has a colon cancer protective effect, partly due to secoisolariciresinol, and that the protective effect is associated with increased β-glucuronidase activity [44].

Anti-tumour effects

In vivo experiments

The effects of linseed oil fed to female mice as 10% of their diet were compared to corn oil, which contains much less α-linolenic acid (18:3n-3) than linseed oil. The respective diets were fed for 3-8 weeks prior to subcutaneous injections of one of two syngeneic mammary tumour cell types (410 and 410.4). The growth of 410.4 mammary tumours was significantly lower in mice given linseed oil than in animals given corn oil ($p<0.05$). Linseed oil also significantly enhanced incorporation of n-3 fatty acids into tumours ($p<0.005$) and significantly reduced tumour prostaglandin E production ($p<0.005$) compared to corn oil. These data suggested an inhibitory effect of dietary α-linolenic acid on mammary tumour growth and metastasis [45].

Supplementation of a high-fat diet (20% corn oil) fed to 70 female rats for 4 weeks with either linseed flour or defatted linseed meal (5% and 10%) reduced epithelial cell proliferation by 38.8-55.4% and nuclear aberrations by 58.8-65.9% in mammary glands, optimum effects being observed with 5% linseed flour [46].

Five groups of 7 male rats were fed the same diet supplemented in the same manner for 4 weeks following a single injection of azoxymethane at 15 mg/kg body weight. In the descending colon of the supplemented groups, the total number of aberrant crypts and foci were significantly reduced ($p\leq0.05$), by 41-53% and 48-57% respectively. The labelling index (the number of labelled cells, or cells undergoing DNA synthesis, per 100 cells in the epithelia of crypts from each section of the rat colon) was 10-22% lower in these groups, except for the 5% linseed meal group [47].

In a long-term experiment, two groups of 60 female rats were fed the same high-fat diet, one group having the diet supplemented with 5% linseed flour. After 4 weeks, tumours were induced in 44 rats by a single dose of 5 mg of 7,12-dimethylbenz[*a*]-anthracene. After an additional week, half of the the group fed the basal diet received the supplemented diet for 20 weeks, while half of the group previously supplemented received the basal diet only, in order to differentiate between initiation and promotional effects of supplementation. The group fed a linseed-supplemented diet only during the promotional stage of mammary carcinogenesis had significantly smaller ($p\leq0.05$) tumour volume (66.7%) than all other groups, but also had an increased tumour burden and number of tumours per group compared with the group fed the supplemented diet throughout the experiment. Feeding the basal diet at the initiation stage of tumour development resulted in a greater number of tumours occurring consistently over time. However, linseed supplementation at initiation and throughout the experimental period tended to reduce the number of tumours per tumour-bearing rat [48].

After administration of a single dose of 5 mg of 7,12-dimethylbenz[*a*]anthracene, 5 groups of female rats ($n = 19$-21) received either a basal diet or a diet supplemented with secoisolariciresinol diglycoside (SD, 2200 nmol/day), 1.82% of linseed oil or 2.5 or 5% of linseed. After 7 weeks of treatment, the volume of established tumours was over 50% smaller in all treatment groups ($p<0.08$, $p<0.04$, $p<0.04$, $p<0.04$ respectively) whereas there was no change in the basal diet group. The number and volume of new tumours were lowest in the SD ($p<0.02$) and 2.5% linseed ($p<0.07$) groups. Combined established and new tumour volumes were smaller in the SD, 2.5 and 5% linseed groups ($p<0.02$) than in the linseed oil and basal diet groups [49].

Antibacterial activity

In vitro experiments

Hydrolysed linseed oil and linolenic acid showed 100% antibacterial activity against methicillin-resistant strains of *Staphylococcus aureus* at concentrations of 0.025% (30°C and 37°C) and 0.01% (37°C) respectively [50].

Pharmacokinetic properties

The breakdown of non-cellulosic polysaccharides takes place mainly in the colon, where anaerobic fermentation yields volatile fatty acids (acetate, propionate and butyrate) and carbon dioxide, hydrogen and methane [51].

Since lignans and isoflavonoid phyto-oestrogens, produced from plant precursors by colonic bacteria, may protect against certain cancers, the effects of ingestion of linseed powder on urinary lignans and isoflavonoids were investigated in 18 premenopausal women in a randomized crossover study. Each consumed her usual omnivorous diet, except avoiding foods containing linseed (high in lignan precursors) or soy (high isoflavone content), for 3 cycles and her usual diet supplemented with linseed (10 g/day) for another 3 cycles, or vice versa. Three-day urine samples from follicular and luteal phases were analysed for lignans and isoflavonoids. Excretion of the two major mammalian lignans, enterodiol and enterolactone, increased with linseed supplementation from 1.09 ± 1.08 and 3.16 ± 1.47 to 19.48 ± 1.10 and 27.79 ± 1.50 μmol/day respectively ($p<0.0002$). Enterodiol and enterolactone excretion in response to

linseed varied widely among the subjects (3- to 285-fold increase). There were no differences in excretion of isoflavonoids or the lignan matairesinol with linseed. Excretion was not altered by phase of the menstrual cycle or duration of linseed consumption [52].

As a sub-study of the above larger study, involving 13 women from the original 18 and the same dietary design, faeces were collected on days 7-11 of the last menstrual cycle in each diet period. Excretion of lignans (nmol/day) increased significantly with linseed intake, from 80.0 ± 80.0 to 2560 ± 3100 for enterodiol ($p<0.01$), from 640 ± 480 to 10,300 ± 7580 for enterolactone ($p<0.01$), and from 7.33 ± 10.0 to 11.9 ± 8.06 nmol/day for matairesinol ($p<0.05$). There were no differences in faecal excretion of isoflavonoids [53].

In a randomized cross-over study, 9 healthy young women supplemented their diets with 5, 15 or 25 g of raw or 25 g of processed (as muffin or bread) linseed for 7 days during the follicular phase of their menstrual cycles. Urine samples (24-hours) were collected at baseline and on the final day of supplementation. As an adjunct to the 25 g raw linseed arm, they consumed 25g for an additional day. Blood and urine samples were collected at specific intervals and analysed for enterolactone and enterodiol. A dose-dependent increase in urinary lignan excretion in response to linseed was observed ($r = 0.72$, $p \leq 0.001$) and processing did not affect the quantity of lignan excretion. Plasma lignan concentrations were significantly greater than baseline ($p \leq 0.001$) by 9 hours after linseed in-gestion (29.35 and 51.75 nmol/litre respectively). The total plasma AUC was higher on the 8th than on the 1st day (1840 and 1027 nmol.h/litre respectively) [54].

Preclinical safety data

No toxic effects of linseed were observed in a brine shrimp lethality bioassay [55].

Linseed did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 102 [55].

Clinical safety data

No drug-related adverse effects were observed from treatment of 70 patients with functional upper abdominal complaint with an aqueous linseed mucilage preparation (1:10) at a dosage of 8 x 25 g (including a small amount of excipients) daily for 3 days [22].

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LIQUIRITIAE RADIX

Liquorice Root

DEFINITION

Liquorice root consists of the dried unpeeled or peeled, whole or cut root and stolons of *Glycyrrhiza glabra* L. It contains not less than 4.0 per cent of glycyrrhizic acid ($C_{42}H_{62}O_{16}$; M_r 823), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

The main characteristic constituents are triterpene glycosides (saponins, 2-15%), principally glycyrrhizic acid, the 3β-diglucuronide of glycyrrhetic acid, which occurs as a mixture of potassium and calcium salts known as glycyrrhizin [2-5].

Other constituents include flavonoids (1-2%) such as liquiritin (a flavanone glucoside) and glabrol (a flavanone); isoflavonoids such as glabrene and glabridin (isoflavans); chalcones such as isoliquiritin; coumarins such as liqcoumarin; polysaccharides and essential oil (approx. 0.05%) [2-5].

Note: The names glycyrrhizic acid and glycyrrhetic acid are used in this text, as in the European Pharmacopoeia. In the literature the names glycyrrhizinic acid and glycyrrhetinic acid respectively are often used for these substances; in some texts the term glycyrrhizin is used synonymously with glycyrrhizic acid.

CLINICAL PARTICULARS

Therapeutic indications

Adjuvant therapy of gastric and duodenal ulcers and gastritis [2,6-11].

Coughs and bronchial catarrh, as an expectorant [2,6-8].

Posology and method of administration

Dosage

Gastric and duodenal ulcers and gastritis

Adult daily dose, taken in divided doses: 5-15 g of liquorice root, equivalent to 200-600 mg of glycyrrhizic acid; equivalent aqueous preparations [2,12] or 5-15 ml of Standardised Liquorice Ethanolic Liquid Extract Ph. Eur. (containing 4.0% m/m of glycyrrhizic acid and 52-65% V/V of ethanol).

Coughs and bronchial catarrh
Adult daily dose, taken in divided doses when required: 1.5-5 g of liquorice root, equivalent to 60-200 mg of glycyrrhizic acid; equivalent aqueous preparations [2,12] or 1.5-5 ml of Standardised Liquorice Ethanolic Liquid Extract Ph. Eur. (containing 4.0% m/m of glycyrrhizic acid and 52-65% V/V of ethanol).

Elderly: dose as for adults.

Children 4 years of age and older as an expectorant only, in aqueous preparations: proportion of adult dose according to age or body weight [13].

Method of administration
For oral administration.

Duration of administration
Preparations of liquorice root should not be taken for more than 4-6 weeks without medical advice [2,7, 14,15].

Contra-indications
Cardiovascular-related disorders such as hypertension [2,7,8,14], renal disorders, cholestatic or inflammatory liver disorders [2,7,14], hypokalaemia [2,7] and severe obesity [14].

Special warnings and special precautions for use
The maximum daily dose of 15 g of liquorice root (or a content of 600 mg of glycyrrhizin) should never be exceeded [15].

Consumption of glycyrrhizin as a taste modifier should be limited to 100 mg/day and, for example, not more than 50 g of liquorice confections (with an average glycyrrhizin content of 0.2%) should be consumed daily [15]. However, individual tolerance varies widely and regular daily intake of even this amount may cause adverse effects in the most sensitive individuals [14,16].

Interaction with other medicaments and other forms of interaction
Hypokalaemia (resulting from excessive use of liquorice root) may potentiate the action of cardiac glycosides and interact with antiarrhythmic drugs or with drugs which induce reversion to sinus rhythm (e.g. quinidine). Concomitant use with other drugs inducing hypokalaemia (e.g. thiazide or loop diuretics, adrenocorticosteroids and stimulant laxatives) may aggravate electrolyte imbalance.

Glycyrrhizic acid has been reported to decrease plasma clearance and increase the AUC of prednisolone [17], and to potentiate hydrocortisone activity in human skin [17,18].

Pregnancy and lactation
Liquorice root should not be used during pregnancy and lactation [2,7].

Effects on ability to drive and use machines
None known.

Undesirable effects
High or prolonged intake may lead to mineralocorticoid effects in the form of electrolyte imbalance (sodium retention and potassium loss) accompanied by hypertension, oedema and suppression of the renin-angiotensin-aldosterone system [2,14,16,19-26]. In rare cases hypokalaemic myopathy may occur [27,28].

Overdose
Prolonged use of excessive doses (preparations equivalent to more than 20 g of liquorice root per day) [14] can cause hypermineralocorticoidism [16,19,21] with various clinical symptoms from hypertension [22-25,29-35], headache [21,36], lethargy, oedema [21] and muscle weakness [30-32] to temporary paralysis [27,32,34], hypertensive encephalopathy and retinopathy [35], and even heart failure or cardiac arrest [2,29,37]. Rare cases of hyperprolactinaemia have also been reported [14,36].

In most cases, after discontinuation of liquorice root the symptoms of overdose revert to normal within a few weeks or several months [8,16].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Inhibitory effects on enzymes
Glycyrrhetic acid inhibited 11β-hydroxysteroid dehydrogenase, an enzyme that converts hydrocortisone (cortisol) to its inactive product cortisone (by converting its 11-hydroxyl group to a ketonic group), in human placental homogenate [38] and rat liver and kidney preparations [39].

Isolated glycyrrhetic acid also potently inhibited Δ^4-5β-reductase activity ($p<0.001$), and to a small extent inhibited Δ^4-5α-reductase activity, in rat liver preparations. Thus it inhibited the inactivation by these two enzymes of Δ^4-3-keto-steroidal hormones such as hydrocortisone (cortisol) and aldosterone through reduction of the C4-C5 double bond to yield dihydro-derivatives, which have no hormonal activity [40-42]. Similar inhibition of Δ^4-5β-reductase was observed in liver preparations from rats to which glycyrrhetic acid or glycyrrhizic acid had previously been administered intramuscularly at 50 mg/100 g body weight for 7 and 14 days respectively [40].

Glycyrrhizic acid was found to be a potent inhibitor of the activity of β-glucuronidase activity in rat liver microsome and a β-glucuronidase-producing strain

of *Escherichia coli* with IC_{50} values of 0.08 and 0.01 mg/ml respectively. 18β-Glycyrrhetic acid was also a potent inhibitor with IC_{50} values of 0.02 and 0.24 mg/ml respectively [43].

Expectorant activity
Due to its saponin content liquorice root decreases surface tension, a mechanism for decreasing the viscosity of mucus and thus increasing secretolytic and expectorant activity. It has no effect on mucociliary transport in the trachea [2,42,44].

Anti-inflammatory and anti-allergic activities
An aqueous extract from *Glycyrrhiza uralensis* root (0.01-1 mg/ml) inhibited tube formation from cultured aortic endothelial cells of rats in the angiogenic phase of the inflammatory process in a concentration-dependent manner (IC_{50}: 0.5 mg/ml). Isolated isoliquiritin had a potency 44-fold greater than that of the liquorice extract and appeared to play a major role in the inhibition of tube formation in angiogenesis; in contrast, glycyrrhizic acid and glycyrrhetic acid increased tube formation [45].

Isolated glycyrrhizic acid had no effect on human neutrophil chemotaxis or phagocytosis. However, it significantly decreased generation of the reactive oxygen species O_2^- (IC_{50}: 0.5 µg/ml), $OH^•$ (IC_{50}: 5 µg/ml) and H_2O_2 (IC_{50}: 5 µg/ml) by human neutrophils [46].

Antiviral activity
When added to infected cultures of human aneuploid HEp2 cells, glycyrrhizic acid (4-8 mM) showed strong inhibitory activity on the growth of vaccinia, herpes simplex, Newcastle disease and vesicular stomatitis viruses [47]. The same compound at 3×10^{-3} M caused complete disappearance of hemagglutinating activity of influenza viruses A and B and Newcastle disease virus in infected embryonated hen eggs [48] and, with an ED_{50} of less than 400 µg/ml, inhibited hepatitis A virus antigen expression in human hepatoma PLC/PRF/5 cells [49].

Glycyrrhizic acid completely inhibited HIV-induced plaque formation in MT-4 cells at a concentration of 0.6 mM (IC_{50}: 0.15 mM). It also completely inhibited the cytopathic effect of HIV and the HIV-specific antigen expression in MT-4 cells at concentrations of 0.3 and 0.6 mM respectively, but had no direct effect on the reverse transcriptase of HIV [50]. Based on subsequent work, the same authors suggested that the inhibitory effect of glycyrrhizic acid on HIV-1 replication may result from a specific inhibition and also from a non-specific inhibition of virus adsorption to the cells complemented by an inhibitory effect on protein kinase C [51].

Antimicrobial effects
Glabrene, glabrol and glabridin showed antimicrobial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* with MICs of 25, 1.56 and 6.25 µg/ml respectively [52].

In vivo experiments

Anti-inflammatory activity
In the adjuvant-induced granuloma pouch test in mice, isolated isoliquiritin administered intraperitoneally at 0.31-3.1 mg/kg body weight dose-dependently inhibited granuloma angiogenesis with an ID_{50} of 1.46 mg/kg, a potency 50-fold greater than that of liquorice root extract (typical isoliquiritin content: 0.8-1.6%), whereas glycyrrhizic acid had an effect weaker than that of the extract; the results indicated that the antiangiogenic effect of liquorice depended on the effect of isoliquiritin. The weight of pouch fluid was also inhibited by isoliquiritin with a potency 18-fold greater than that of the extract [45].

Antiulcerogenic effects
Granules of ibuprofen (60 mg/kg), alone or coated with liquorice containing more than 6.8% of glycyrrhizic acid or deglycyrrhized liquorice or highly glycyrrhized liquorice containing 15% of glycyrrhizic acid (30 mg/kg of coating in each case), were administered orally to rats as a single dose and mucosal damage was assessed 4 hours later. Compared to treatment with ibuprofen only, the additional liquorice extract ($p<0.05$) or deglycyrrhized liquorice ($p<0.02$) significantly reduced the size and number of mucosal lesions and ulcers in the rat gastric mucosa; highly glycyrrhized liquorice was less effective [53]. In a similar experiment with aspirin at 266 mg/kg, coating with liquorice, deglycyrrhized liquorice or highly glycyrrhized liquorice (133 mg/kg of coating in each case) significantly reduced the ulcer index ($p<0.001$ in each case), liquorice being the most effective in reducing the incidence of ulcers (46% incidence compared to 96% with aspirin alone) [54].

A liquid alcoholic extract of liquorice root (16 mg of glycyrrhizic acid per ml), administered orally to rats at 2.5-10 ml/kg body weight, had a histologically-confirmed, dose-dependent protective effect against indometacin-induced ulcers; about 95% protection at 10 ml/kg was comparable to the effect of oral cimetidine at 100 mg/kg. Oral pre-treatment of rats with the same extract (but lyophilized and reconstituted with water only) at 5 ml/kg significantly reduced gastric juice acidity, increased mucin concentration, and increased the prostaglandin E_2 content but reduced the leukotriene content of the gastric juice compared to indometacin-treated animals [55].

A decoction from 2 g of liquorice root, orally administered to rats daily for 15 days, did not significantly reduce total gastric acid secretion but significantly lowered free hydrochloric acid ($p<0.05$) by about 50% compared to the control group [11].

Effect on pancreatic secretion
Intraduodenal administration to dogs of a dry fraction from a methanolic extract of liquorice root containing 13-19% of glycyrrhizic acid and 4-13% of isoflavonoids at three different doses (0.5, 1 and 2 g) induced significant increases in both plasma secretin concentration and pancreatic bicarbonate secretion in a dose-related manner. Intragastric administration of the liquorice fraction (2 g) in 5% liver extract meal also resulted in significant increases in both plasma secretin levels and pancreatic bicarbonate output [42,56].

Anti-tumour activity
Initiation with 7,12-dimethylbenzen[*a*]anthracene (DMBA, one application) followed by promotion with 12-*O*-tetradecanoylphorbol-13-acetate (TPA, twice weekly for 15 weeks) of tumours on the depilated skin of mice were significantly inhibited by topical pretreatment with 18α- or 18β-glycyrrhetic acid (GA) over a 16-week period. As total number of tumours per mouse, α-GA pretreatment resulted in 20% inhibition ($p<0.05$) and β-GA in 50% inhibition ($p<0.001$) [57].

Hepatoprotective activity
Glycyrrhizic acid administered orally to rats at 100 mg/kg had a preventive effect, attributed to its β-glucuronidase-inhibiting activity, on carbon tetrachloride-induced hepatotoxicity ($p<0.01$). When glycyrrhizic acid was administered intraperitoneally at 100 mg/kg no significant effect was observed, but glycyrrhetic acid showed significant hepatoprotective activity at 50 mg/kg ($p<0.05$) and 100 mg/kg ($p<0.01$) [43].

Pharmacological studies in humans
Daily doses of dried aqueous extract of liquorice root containing 108, 217, 380 or 814 mg of glycyrrhizic acid were administered for 4 weeks to similar groups 1, 2, 3 and 4 respectively of healthy volunteers, each comprising 3 males and 3 females (aged from 22 to 39 years). Parameters evaluated before the study and after 1, 2 and 4 weeks involved anthropometric measurements, heart rate, mean arterial pressure, renal function, serum electrolytes, renin-aldosterone axis, blood glucose and haematocrit. No significant effects were observed in groups 1 and 2. Depression of plasma renin activity occurred in groups 3 ($p = 0.024$) and 4 ($p = 0.049$), and of plasma aldosterone concentration in group 4 ($p = 0.019$). In group 4, transient reduction in kalaemia ($p = 0.014$) and increase in body weight ($p = 0.041$) were noted after 1 and 2 weeks respectively, but had returned towards the baseline by the end of week 4.

Administration was stopped after 2 weeks in three subjects due to side effects: a female in group 3 (continuous headache), a male in group 4 with a family history of hypertension (arterial hypertension) and a female in group 4 who was also taking an oral contraceptive (headache, borderline arterial hypertension, hypokalaemia and peripheral oedema). In each case, the side effects disappeared within 24-48 hours after discontinuation.

Overall, the intake of up to 217 mg of glycyrrhizic acid per day (as liquorice extract) led to neither clinical effects nor changes in laboratory parameters, suggesting the absence of mineralocorticoid-like activity. Only the two highest doses of extract led to adverse effects in healthy subjects, in particular those with subclinical diseases or in situations favouring sodium retention, such as in the premenstrual period or when taking oral contraceptives. The effects were less common and less pronounced than those reported after comparable intake of isolated glycyrrhizic acid, as such or as a flavouring in food. Clinical symptoms disappeared promptly on discontinuation of the extract. The metabolic effects of liquorice root extract were dose-related and more frequent in women than in men [58].

In another study, 14 healthy volunteers ate 100 g or 200 g of confectionery liquorice (equivalent to 0.7-1.4 g of glycyrrhizic acid) daily for 1-4 weeks. Plasma potassium levels fell by over 0.3 mM in 11 subjects, including 4 who had to be withdrawn from the study because of hypokalaemia. Sodium retention, with concomitant weight gain was also evident, but blood pressure did not rise significantly in any of the subjects. One or more values of the renin-angiotensin-aldosterone axis, especially plasma renin activity and urinary aldosterone levels, were considerably depressed in all subjects. The results demonstrated that potentially serious metabolic effects may occur in some persons after intake of modest amounts of liquorice daily for less than one week [21].

Glycyrrhetic acid, administered orally to 10 healthy, normotensive volunteers for 8 days at 500 mg/day (in two divided doses), exerted pronounced mineralocorticoid activity as shown by significant increases in plasma sodium ($p<0.01$) and urinary potassium, significant decreases in plasma potassium ($p<0.01$) and aldosterone ($p<0.05$), and changes in other parameters. Urinary excretion of free hydrocortisone was elevated and plasma hydrocortisone levels virtually unchanged in the presence of markedly decreased levels of both plasma cortisone and urinary free cortisone. The results provided direct clinical support for the hypothesis that glycyrrhetic acid induces inhibition of the activity of 11β-hydroxysteroid dehydrogenase, resulting in a blockade in the conversion of hydrocortisone (cortisol) to cortisone [20].

Clinical studies

Anti-ulcer activity
In an open study, 15 patients with radiologically proven peptic ulcer were treated with 3 × 3 g of powdered

liquorice root daily for 1-3 months. Evaluation after 2 months suggested that liquorice root produced beneficial effects, relieving pain in the epigastrium (56% of cases) and burning in the epigastric/retrosternal regions (78% of cases). Radiological evidence showed complete or near complete healing in 50% of cases and partial healing in a further 40% [11].

Although some studies using deglycyrrhizinated liquorice showed apparent beneficial effects on ulcers [59], placebo-controlled studies involving a total of 271 patients and daily dosages of 2.3-5 g of deglycyrrhizinated liquorice for 4-6 weeks revealed no clinical advantage over placebo in the treatment of gastric [60,61] or duodenal [62,63] ulcers.

Pharmacokinetic properties

Pharmacokinetics in vitro

Under anaerobic conditions, human intestinal bacteria hydrolyzed glycyrrhizic acid to its aglycone, glycyrrhetic acid, which was then isomerized through 3-dehydro-glycyrrhetic acid to 3-*epi*-glycyrrhetic acid and *vice versa* [64,65].

Pharmacokinetics in animals

The mechanism of gastrointestinal absorption of glycyrrhizic acid in rats was investigated using an *in situ* loop technique. Glycyrrhizic acid was poorly absorbed from the gut of rats (with a bioavailability of about 4%), but after oral administration at 200 mg/kg it was detected in rat plasma together with glycyrrhetic acid. Glycyrrhizic acid was hydrolysed to glycyrrhetic acid by rat gastric and large-intestinal (but not small-intestinal) contents. The glycyrrhetic acid formed was absorbed mainly from the large intestine, with a bioavailability of 14% and a peak plasma concentration 12 hours after oral dosing. After intravenous administration of glycyrrhizic acid, no glycyrrhetic acid could be detected in the plasma, suggesting that systemic hydrolysis is low, and 80% of the dose was excreted unchanged in the bile [66]. When glycyrrhizic acid (100 mg/kg) was administered orally to germ-free rats, no glycyrrhetic acid was detected in the plasma, caecal contents or faeces, indicating that hydrolysis to glycyrrhetic acid depends on bacteria [67].

After bolus injection of glycyrrhizic acid into the portal vein, its plasma level fell rapidly within 30 minutes, but at 60 minutes it had fallen only slightly further. This suggested that glycyrrhizic acid is distributed in the tissues then eliminated from the blood and excreted only slowly [68]. From studies on enterohepatic recycling of glycyrrhizic acid in rats following intravenous administration (100 mg/kg) it was concluded that glycyrrhizic acid was predominantly secreted from the liver into the bile (80.6 ± 9.9% of the administered dose) [69].

The effects of other components of aqueous liquorice root extract on the pharmacokinetics of glycyrrhizic acid and glycyrrhetic acid were investigated in rats. Lower plasma levels of glycyrrhizic acid and glycyrrhetic acid were found in rats treated orally with the aqueous extract compared to those treated with a corresponding amount of pure glycyrrhizic acid [70,71]. After complete removal of glycyrrhizic acid from a liquorice root extract and separation of the remainder into lipophilic and hydrophilic components, the lipophilic components were found to reduce absorption of glycyrrhizic acid; in contrast, the hydrophilic components increased the bioavailability of glycyrrhizic acid as glycyrrhetic acid [72].

Pharmacokinetics in humans

Plasma levels of glycyrrhetic acid after oral administration of an aqueous liquorice root extract to healthy volunteers were found to be lower than those observed after oral administration of a corresponding amount of pure glycyrrhizic acid [70,71]. The lower absorption and bioavailability from liquorice root extract could explain the various adverse clinical effects resulting from chronic oral administration of pure glycyrrhizic acid as opposed to the extract [70].

After oral administration of a liquorice root decoction containing 133 mg of glycyrrhizic acid to 5 healthy volunteers the peak serum concentration of glycyrrhizic acid occurred less than 4 hours after administration and was not detectable after 96 hours. In contrast, the serum concentration of glycyrrhetic acid reached a maximum after about 24 hours and remained detectable in the urine for approx. 130 hours. The low urinary concentrations of both compounds suggested excretion via the gastrointestinal route [73]. In another study, after oral administration of 100 mg of glycyrrhizic acid to 3 healthy volunteers none was detected in plasma but 1-3% was detected in the urine, suggesting partial absorption intact from the gastrointestinal tract; glycyrrhetic acid appeared in the plasma at < 200 ng/ml but was not detected in the urine [74].

A comparison study in which glycyrrhizic acid was administered orally to 10 healthy men before or after breakfast showed that maximum plasma concentrations and areas under curves of glycyrrhizic acid and glycyrrhetic acid were not significantly influenced by consumption of food [75].

The glycyrrhetic acid level in blood of 10 patients with, and 11 patients without, liquorice-induced pseudoaldosteronism was comparable. On the other hand, the level of another metabolite, 3β-monoglucuronyl-glycyrrhetic acid, was elevated in patients with pseudoaldosteronism but not in patients without this syndrome. It is likely that in certain patients, especially older ones, longer administration of glycyrrhizic acid may induce an enzyme in intestinal

bacterial flora which results in increased production of 3β-monoglucuronyl-glycyrrhetic acid and that this metabolite causes pseudoaldosteronism [76].

Preclinical safety data

Acute toxicity

For a liquorice root dry extract containing 48-58% of glycyrrhizic acid the oral, intraperitoneal and subcutaneous LD_{50} values in rats and mice were determined as 14.2-18.0 g/kg, 1.42-1.70 g/kg and 4.0-4.4 g/kg respectively [77]. The oral LD_{50} of glycyrrhetic acid was determined as 560 mg/kg in mice [41].

Subacute toxicity

Oral administration of a liquorice root dry extract containing 48-58% of glycyrrhizic acid to rats at 0.31-0.63 g/kg/day for 90 days had no toxic effect, whereas 2.5 g/kg/day over the same period led to decreases in body weight gain, blood cell count and thymus weight, and also atrophic cortex and sporadic lymphofollicle formation in the medulla of the thymus gland. All these changes disappeared after discontinuation of the drug [77].

Subchronic toxicity

Rats fed on a diet containing 1.2-2.6 g/kg/day of the ammonium salt of glycyrrhizic acid for 4-6 months exhibited hypertension, increased relative weights of kidney and heart, and a slight decrease in body weight and growth as well as bradycardia and polydipsia [78].

Mutagenicity and carcinogenicity

An aqueous extract from liquorice root and various fractions from it showed no mutagenic potential in the Ames test using *Salmonella typhimurium* strains TA98 and TA100 [79]. In the *Salmonella*/microsome reversion assay using strain TA100 an ethanolic extract of liquorice root (0.8% glycyrrhizic acid) showed antimutagenic activity against ethyl methanesulphonate and ribose-lysine [80].

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LUPULI FLOS

Hop Strobile

DEFINITION

Hop strobile consists of the dried, generally whole, female inflorescences of *Humulus lupulus* L.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

Bitter principles consisting mainly of prenylated phloroglucinol derivatives called α-acids or humulones (2-12% of dried strobile), principally humulone (35-70%), and β-acids or lupulones (1-10% of dried strobile), principally lupulone (30-55%) [2-6].

Essential oil, 0.5-1.5% [2] consisting mainly of myrcene, humulene and β-caryophyllene [2,7]. Although only a trace of 2-methyl-3-buten-2-ol is found in freshly-harvested hop strobile [8,9], the amount is higher in stored material, increasing to a maximum of approx. 0.15% of the dry weight (up to 20% of the volatiles) after 2 years due to degradation of humulones and lupulones [4,5,8,9].

Flavonoids (0.5-1.5%) including quercetin and kaempferol glycosides [10,11] and at least 22 prenylated (or geranylated) flavonoids [12], notably the chalcones xanthohumol (up to 1% of dried strobile and 80-90% of total flavonoids), desmethyl-xanthohumol and dehydrocycloxanthohumol, and the flavanones isoxanthohumol, 8-prenylnaringenin (25-60 mg/kg) and 6-prenylnaringenin [12-16].

Other constituents include proanthocyanidins, phenolic acids, proteins (15%), polysaccharides (40-50%) and minerals [2,3,5].

CLINICAL PARTICULARS

Therapeutic indications

Tenseness, restlessness and sleep disorders [4,17-20].

Posology and method of administration

Dosage

Internal use

Adults and children over 12 years of age: 0.5 g of the drug as an infusion, 2-4 times daily; 0.5-2 ml of liquid extract (1:1, 45% ethanol) or 1-2 ml of tincture (1:5,

60% ethanol), up to 3 times daily; other equivalent preparations [19,20].

External use
Infants and young children: Up to 500 g of dry hop strobile (previously stored for 1-2 years) in a hop pillow [21].

Method of administration
For oral administration; combination with other herbal sedatives may be beneficial [4]. Also externally in hop pillows for overnight use [21].

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, hop strobile preparations should not be used internally during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None reported.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Over the past decade considerable pharmacological research has been carried out on hop strobile and its constituents, particularly with respect to oestrogenic activity. However, no new studies relating to the sedative effects of hop strobile appear to have been published.

In vitro experiments

Oestrogenic activity of hop strobile
Circumstantial evidence over many years, including menstrual disturbances reported to be common among female hop pickers, linked hop strobile with potential oestrogenic activity [2,3]. However, early studies to confirm this activity experimentally were inconclusive or contradictory due to methodology of inadequate sensitivity [3,22].

In recent screening of plant drugs for oestrogenic activity, a 50%-ethanolic extract (2 g of hop strobile to 10 ml) exhibited binding to oestrogen receptors in intact, oestrogen-dependent [ER(+)], human breast cancer MCF-7 cells with a potency equivalent to 0.5 µg of oestradiol per 2 g of dried strobile (for comparison, the potencies of 2 g of thyme or red clover were equivalent to 0.5 or 3 µg of oestradiol, respectively). The extract also showed significant ability to *stimulate* cell proliferation in ER(+) T47D, but not in ER(–) MDA 468, breast cancer cells [23]. In contrast, in a different series of experiments, a similarly-prepared extract of hop strobile at concentrations of 0.01-1.0% V/V was found to significantly *inhibit* serum-stimulated growth of ER(+) T47D breast cancer cells ($p<0.001$) [24].

Ovarian cells isolated from immature female rats, which 48 hours previously had been injected (primed) with pregnant mare's serum gonadotrophin, were incubated with follicle-stimulating hormone to induce oestradiol secretion. Addition to the culture medium of purified water-soluble fractions F_1 or F_2 from de-fatted hop strobile extract reduced the amounts of oestrogen E_2 released from the ovarian cells ($p<0.01$) with a probably related decrease in cAMP release ($p<0.05$) [25].

Oestrogenic activity of 8-prenylnaringenin
8-prenylnaringenin, a flavanone occurring in hop strobile at levels of 25-60 mg/kg [15], has been shown to be a potent phyto-oestrogen with activity greater than that of other established plant oestrogens [26]. Oestrogenic activity of a much lower order (less than one-hundredth of that of 8-prenylnaringenin) has also been detected in three other hop flavonoids, 6-prenylnaringenin, 8-geranylnaringenin and 6,8-diprenylnaringenin, while xanthohumol and isoxanthohumol were found inactive [16]. EC_{50} values for 17β-oestradiol, 8-prenylnaringenin, 6-prenylnaringenin, coumestrol, genistein and daidzein were 0.3, 40, >4000, 70, 1200 and 2200 nM, respectively, in a screen using oestrogen-inducible yeast (*Saccharomyces cerevisiae*) expressing the human oestrogen receptor, and 0.8, 4, 500, 30, 200 and 1500 nM, respectively, for stimulation of alkaline phosphatase activity in a human endometrial cell line (Ishikawa Var I). The relative binding affinities of 17β-oestradiol, 8-prenylnaringenin, coumestrol and genistein with rat uterine cytosol containing soluble oestrogen receptor were 1, 0.023, 0.008 and 0.003, respectively [26].

The oestrogenic activity of 8-prenylnaringenin was confirmed in competitive binding assays using purified human recombinant oestrogen receptors α and β (ERα and ERβ). 8-prenylnaringenin competed strongly with 17β-oestradiol for binding to both receptors with

a relative binding affinity of about 0.1, compared to 1.0 for 17β-oestradiol and 0.001 for 8-geranylnaringenin [16].

In another study, involving displacement of [^3H]-17β-oestradiol, 8-prenylnaringenin showed competitive binding affinity for the oestrogen receptor in bovine uterine cytosol with an IC_{50} of 140 nM, compared to 1.0 nM for oestradiol and 320 nM for genistein. 8-Prenylnaringenin also dose-dependently stimulated the proliferation of cultured, oestrogen-dependent, human breast cancer MCF-7 cells with an EC_{50} of 1.9 nM, compared to 0.0032 nM for oestradiol and 47 nM for genistein, suggesting that it was an oestrogen receptor agonist [27].

Antiproliferative activity of xanthohumols
Xanthohumol (XN), dehydrocycloxanthohumol (DX) and isoxanthohumol (IX) caused dose-dependent (0.1 to 100 μM) decreases in growth of human breast cancer MCF-7, colon cancer HT-29 and ovarian cancer A-2780 cells, xanthohumol being the most potent. With MCF-7 cells the IC_{50} values were 13.3, 15.7 and 15.3 μM after 2 days and 3.5, 6.7 and 4.7 after 4 days for XN, DX and IX, respectively. HT-29 cells were more resistant than MCF-7 cells. With A-2780 cells xanthohumol was highly antiproliferative with IC_{50} values of 0.5 and 5.2 μM after 2 and 4 days of exposure, respectively. At 100 μM all three compounds were cytotoxic to all three cell lines [28].

Spasmolytic activity
An alcoholic extract of hop strobile (1 g of dried drug in 10 ml of 70 % ethanol) produced a strong spasmolytic effect on isolated smooth muscle from guinea pig intestine with ED_{50} values equivalent to 37×10^{-6} g of hop strobile per ml for acetylcholine-induced contractions compared to 60×10^{-9} g/ml with atropine, and 39×10^{-6} of hop strobile per ml for barium chloride-induced contractions compared to 57×10^{-7} g/ml with papaverine. The extract also inhibited contractions of rat uterus with an ED_{50} equivalent to 31×10^{-6} g of hop strobile per ml [29].

Effect on calcium flux
A methanolic extract from hop strobile showed strong inhibitory activity on calcium fluxes, inhibiting depolarization-induced $^{45}Ca^{2+}$ uptake in clonal rat pituitary cells by 94.7% at 20 μg/ml ($p<0.001$). The activity was attributed to prenylated flavonoids, although individual compounds from hop strobile have not so far been tested in this way [30].

Other activities
A methanolic extract of hop strobile showed inhibitory activity against rat liver diacylglycerol acyltransferase (DGAT), which is involved in triacylglycerol formation. By fractionation, the activity was traced to xanthohumol and a related chalcone, xanthohumol B; they inhibited DGAT activity in rat liver microsomes dose-dependently with IC_{50} values of 50 and 194 μM, respectively, and also inhibited DGAT activity in intact Raji cells [31].

Using an *in vitro* 'pit formation assay' (formation of pits on dentine slices incubated with mouse bone cells), xanthohumol and humulone have been identified as inhibitors of bone resorption at concentrations at or above 10^{-6} and 10^{-11}, respectively ($p<0.01$); humulone showed remarkably high inhibitory activity with an IC_{50} of 5.9×10^{-9} M [32].

Several prenylated flavonoids from hop strobile, particularly xanthohumol, isoxanthohumol and 8-prenylnaringenin, were shown to be potent and selective inhibitors of certain cDNA-expressed human cytochrome P450 enzymes known to bioactivate carcinogens. At 10 μM, xanthohumol almost completely inhibited (2.5% of control) the 7-ethoxyresorufin O-deethylase (EROD) activity of CYP1A1 and completely eliminated EROD activity of CYP1B1, while other prenylated flavonoids showed somewhat less activity. In contrast, 8-prenylnaringenin (25 μM) and isoxanthohumol (100 μM) were the most effective inhibitors of CYP1A2 acetanilide 4-hydroxylase activity (> 90% inhibition) and also of CYP1A2 metabolism of the carcinogen aflatoxin B_1 [33].

In vivo experiments

Sedative effects
To assess effects on the central nervous system, an extract of hop strobile was administered intraperitoneally to mice before a series of behavioural tests. Spontaneous locomotor activity was dose-dependently suppressed by 100 mg/kg ($p<0.05$), 250 and 500 mg/kg ($p<0.001$); at 250 and 500 mg/kg the activity was 11% and 3% respectively of that of saline-treated mice in the first hour after administration. Pentobarbital-induced sleeping time increased dose-dependently; not significant at 100 mg/kg, by 1.9-fold at 250 mg/kg ($p<0.05$) and 2.6-fold at 500 mg/kg ($p<0.01$). In the hot plate test, latency time for licking the forepaws increased with doses of 100 and 250 mg/kg ($p<0.01$). Rotarod performance decreased by 59% and 65% respectively at 250 and 500 mg/kg ($p<0.05$). The time to onset of convulsion and survival time after administration of pentylenetetrazole were significantly lengthened by 500 mg/kg ($p<0.001$), but not by 250 mg/kg. A significant and time-dependent fall in rectal temperature was observed after a dose of 500 mg/kg ($p<0.001$ after 120 minutes). Thus hop strobile extract showed sedative and hypnotic properties at lower doses (100-250 mg/kg), and at a higher dose of 500 mg/kg it also produced anticonvulsive and hypothermic effects [34,35].

2-Methyl-3-buten-2-ol, given intraperitoneally to mice

at a high dose of 800 mg/kg, showed central nervous depressant activity, producing deep narcosis for about 8 hours, without subsequent abnormal behaviour [36]; in rats, intraperitoneal administration at 206.5 mg/kg caused a decline in motility of 50% [37]. Although 2-methyl-3-buten-2-ol is present only in small amounts in hop strobile, higher levels may be generated in vivo by metabolism of humulones and lupulones [8,9,38]. The sedative effect of this constituent is comparable, in the same dosage range, to that of the structurally-related drug methylpentynol [37].

Antigonadotrophic effects

Purified water-soluble fractions from de-fatted hop strobile extract were administered subcutaneously twice daily for 3 days to immature female rats primed with 25 IU of pregnant mare's serum gonadotrophin (PMSG). None of the fractions induced a change in uterine weights. However, fractions F_1 (20 mg/rat) and F_2 (50 mg/rat) significantly suppressed PMSG-induced gain in ovarian weights by about 25% ($p<0.05$) compared to controls. Under the same conditions, two further fractions (4 mg/rat) purified from F_1 suppressed gain in ovarian weights by 42% and 33% ($p<0.01$) compared to controls [39]. In further experiments on PMSG-primed immature rats, by comparison with saline-treated control animals, subcutaneously administered fractions F_1 and F_2 reduced the number of ovulations ($p<0.05$); suppressed levels of serum luteinizing hormone ($p<0.001$); suppressed thymidine kinase activity in uterine tissue ($p<0.01$); reduced oestradiol E_2 secretion in cultures of ovarian cells from the rats ($p<0.001$); and reduced progesterone production in cultures of luteal cells from the rats ($p<0.05$ to $p<0.001$) [25].

Oestrogenic effects of 8-prenylnaringenin

Ovariectomized rats, as a model for oestrogen deficiency-induced osteoporosis, were treated subcutaneously with racemic 8-prenylnaringenin (OVX + 8PN) at 30 mg/kg/day or with 17β-oestradiol (OVX + OE) at 0.01 mg/kg/day, or with vehicle only (OVX). Another group of rats was sham-operated, i.e. subjected to ovariectomy surgery without removing the ovaries, and treated with the vehicle only (sham). After 2 weeks of treatment, 24-hour urine samples were collected and body weight gain, uterine weight and bone mineral density were determined. The uterine weights of sham and OVX + 8PN rats were found to be 165% higher ($p<0.001$), and of OVX + OE rats 235% higher ($p<0.001$), than those of OVX rats. Body weight gains of sham, OVX + OE and OVX + 8PN were significantly lower ($p<0.05$) than those of OVX rats. Urinary excretion of hydroxyproline (a conventional marker of bone resorption) was 1.62 µg/g/day from OVX rats compared to 1.18 and 1.16 µg/g/day for sham and OVX + OE rats respectively ($p<0.01$), and 1.01 µg/g/day from OVX + 8PN rats ($p<0.001$). The levels of urinary hydroxypyridinium crosslinks (assayed as pyridinoline and deoxypyridinoline), which are recognized to be directly related to bone matrix degradation, were significantly lower in sham rats ($p<0.01$), and in OVX + OE and OVX + 8PN rats ($p<0.001$), than in OVX rats. Bone mineral densities of 139.1, 141.9 and 141.9 mg/cm² in sham, OVX + OE and OVX + 8PN rats, respectively, were significantly higher ($p<0.001$) than that in OVX rats (132.1 mg/cm²). It was concluded that 8-prenylnaringenin functions as an oestrogen receptor agonist in reproductive tissues and that the dosage used had completely prevented ovariectomy-induced bone loss [40].

It should be noted, however, that (as summarized in the *in vitro* section) xanthohumol and especially humulone, neither of which has oestrogenic activity, also appear to be inhibitors of bone resorption [32]. If this finding can be corroborated, it may indicate that the inhibition of bone resorption is not associated with oestrogenicity [3].

Anti-inflammatory effects

A dry methanolic extract of hop strobile, applied topically at 2 mg/ear, inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear oedema in mice by 90% ($p<0.01$) six hours after TPA treatment. Humulone, isolated from hop strobile by bioassay-guided fractionation and identified as an anti-inflammatory constituent, inhibited the oedema with an ID_{50} of 0.2 mg/ear (ID = inhibitory dose) [41]. Topically-applied humulone also inhibited arachidonic acid-induced inflammatory ear oedema in mice with an ID_{50} of 2.2 mg/ear ($p<0.01$ against controls) compared to 0.4 mg/ear ($p<0.01$) for indometacin [42].

Inhibition of tumour promotion

Humulone applied topically at 1 mg/mouse to the backs of mice markedly inhibited the tumour-promoting effect of TPA on 7,12-dimethylbenz[*a*]-anthracene-initiated skin tumour formation. In the control group 100% of mice developed tumours (first tumour appeared in week 6), compared to only 7% in the humulone-treated group (first appearance in week 16). Humulone treatment resulted in a 99% reduction in the average number of tumours per mouse at week 18 ($p<0.01$) [42].

Clinical studies

In a placebo-controlled study, 20 patients experiencing hot flushes due to ovarian insufficiency (15 in the menopausal phase and 5 following ovariectomy) were treated with a dry aqueous extract of hop strobile (5:1), initially at 1.6-2.6 g/day, later reduced in some cases to 1.2-1.6 g/day. 5 other patients received placebo. Assessment was based on scores calculated by multiplying the intensity of hot flushes (scale of 1 to 3) by their frequency (scale of 1 to 9). In verum patients, the initial average score of 22.7 decreased to 8.2 after 30 days of treatment, whereas

in the placebo group the initial score of 20 decreased only to 18. Compared to the placebo group, 76% of the verum patients achieved a statistically significant improvement in scores and 7 out of 20 patients achieved a reduction in score of at least 15 points [43].

Pharmacokinetic properties
No data available.

Preclinical safety data
Toxicity data on hop strobile are unavailable but, as an ingredient extensively used in the brewing industry, it is generally considered to lack toxicity.

In the Ames mutagenicity test, a hydroethanolic extract of hop strobile showed weakly mutagenic potential in *Salmonella typhimurium* strains TA 98 and TA 100, with or without activation [44].

Oestrogenic effects of hop strobile, and particularly of the potent constituent 8-prenylnaringenin, have been demonstrated, as summarized above. If hop strobile or extracts were consumed in sufficient amount, these effects could potentially be beneficial or undesirable depending on the circumstances. The content of 8-prenylnaringenin has been determined as 26-58 ppm in hop strobile, 1-13 ppm in carbon dioxide extracts of hop strobile used in brewing, 0.009-0.021 ppm in ales, and undetectable to 0.009 ppm in lagers [15]. Subcutaneous administration of 8-prenylnaringenin to rats for 2 weeks at 30 mg/kg/day produced no overt signs of toxicity [40].

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MATRICARIAE FLOS

Matricaria Flower

DEFINITION

Matricaria flower consists of the dried flower-heads of *Matricaria recutita* L. [*Chamomilla recutita* (L.) Rauschert]. It contains not less than 4 ml/kg of blue essential oil.

The material complies with the monograph of the European Pharmacopoeia [1].

Fresh material may also be used provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

The main characteristic constituents of matricaria flower are the essential oil (0.5-1.5%) and flavone derivatives [2-6] such as apigenin-7-glucoside (approx. 0.5%) [2].

The essential oil contains approximately 50% of the sesquiterpenes (–)-α-bisabolol and its oxides A, B and C [6], bisabolonoxide A, up to 25% of *cis*- and *trans*-en-yn-dicycloethers (or spiroethers) [2] and matricin, which is converted to chamazulene on distillation (up to 15%) [2].

Other constituents of matricaria flower include coumarins (herniarin and umbelliferone) [2-4,6], phenolic acids [2,3,6] and polysaccharides (up to 10%) [2,3,6,7].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Symptomatic treatment of gastrointestinal complaints such as minor spasms, epigastric distension, flatulence and belching [2,5,8-14].

External use

Minor inflammation and irritations of skin and mucosa, including the oral cavity and the gums (mouth washes), the respiratory tract (inhalations) and the anal and genital area (baths, ointments) [8,11,14-29].

E/S/C/O/P
MONOGRAPHS
Second Edition

Posology and method of administration

Dosage

Internal use
Adults: As a tea infusion: 3 g of the drug to 150 ml of hot water, three to four times daily.
Fluid extract (1:2; 50% ethanol as preferred extraction solvent): 3-6 ml daily [2,30].
Dry extract: 50-300 mg three times daily [31].
Elderly: dose as for adults.
Children: Proportion of adult dose according to age or body weight.

External use
For compresses, rinses or gargles: 3-10% m/V infusion or 1% V/V fluid extract or 5% V/V tincture [2,31].
For baths: 5 g of the drug, or 0.8 g of alcoholic extract, per litre of water [2].
For solid and semi-solid preparations: hydroalcoholic extracts corresponding to 3-10% m/m of the drug [4,5].
For vapour inhalation: 10-20 ml of alcoholic extract per litre of hot water [28].

Method of administration
For oral administration, local application and inhalation.

Duration of administration
No restriction.

Contra-indications
Sensitivity to *Matricaria* or other members of the Compositae.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No harmful effects reported.

Effects on ability to drive and use machines
None known.

Undesirable effects
Rare cases of contact allergy have been reported in persons with known allergy to *Artemisia* species [6]. Matricaria flower of the bisabolol oxide B-type can contain traces of the contact allergen anthecotulide [6,14,32]. Matricaria possesses a much lower allergenic potential than other chamomile species and therefore allergic reactions to matricaria must be considered as extremely rare. Most of the described allergic reactions to matricaria were due to contamination with *Anthemis cotula* or related species, which contain high amounts of anthecotulide. However, in cases where matricaria contact allergy has been acquired, cross-reactions to other sesquiterpene lactone-containing plants are common [32,33].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Anti-inflammatory effects

In vitro experiments
Ethanolic (48% V/V) and isopropanolic (48% V/V) extracts of matricaria flower inhibited 5-lipoxygenase, cyclooxygenase and the oxidation of arachidonic acid with IC_{50} values of 0.05-0.3%, while a super-critical carbon dioxide extract had an IC_{50} of 6-25 µg/ml for these activities [34]. Investigation of individual constituents revealed that apigenin inhibited 5- and 12-lipoxygenase (IC_{50}: 8 and 90 µM respectively); chamazulene and (–)-α-bisabolol inhibited only 5-lipoxygenase (IC_{50}: 13 and 40 µM respectively); apigenin, *cis*-en-yn-spiroether and (–)-α-bisabolol inhibited cyclooxygenase (IC_{50}: 70-80 µM); only chamazulene had antioxidative activity (IC_{50}: 2 µM) [34].

Trans-en-yn-dicycloether inhibited the provoked degranulation of rat mast cells in concentrations above 0.1 mM [35].

Apigenin markedly inhibited the transcriptional activation of cyclooxygenase (IC_{50}: 8.7 µM) and of nitric oxide synthase (IC_{50}: 3.1 µM) in lipo-polysaccaride-activated macrophages [36].

In vivo experiments
The anti-inflammatory effects of orally administered (–)-α-bisabolol have been demonstrated in carrageenan-induced rat paw oedema, adjuvant arthritis of the rat, ultraviolet-induced erythema of the guinea pig and yeast-induced fever of the rat [37]. In the carrageenan-induced rat paw oedema test the following ED_{50} values (mmol/kg) were obtained after oral administration: (–)-α-bisabolol 2.69, chamazulene 4.48, guaiazulene 4.59, matricin 2.69 and salicyl-amide 1.53 [38].

A dry extract prepared from infusion of 20 g of matricaria flower in 100 ml of 42% ethanol, applied topically at 750 µg/ear, inhibited croton oil-induced oedema of mouse ear by 23.4% compared to controls; benzydamine at 450 µg/ear showed comparable inhibition of 26.6% [39]. In the same test system, two polysaccharides from matricaria flower at 300 µg/ear

inhibited oedema by 14% and 22% respectively [40].

Antispasmodic effects

In vitro experiments
A hydroethanolic extract of matricaria flower showed antispasmodic activity on isolated guinea pig ileum stimulated by various spasmogens. The ED_{50} (mg/ml) and the strength of activity relative to papaverine (= 1.0) respectively were 1.22 and 0.0011 with barium chloride, 1.15 and 0.0019 with histamine dihydrochloride, 2.24 and 0.00074 with bradykinin, and 2.54 and ca. 0.00062 with serotonin. Pure constituents were also investigated: with barium chloride, (–)-α-bisabolol (ED_{50}: 136 µg/ml) exhibited activity comparable to papaverine while apigenin (ED_{50}: 0.8 µg/ml) was more than 3 times as active [41].

Anti-ulcerogenic effect

In vivo experiments
The development of ulcers induced in rats by indometacin, stress or ethanol was inhibited by an orally administered extract of matricaria flower with an ED_{50} of 1 ml per rat and by (–)-α-bisabolol with an ED_{50} of 3.4 mg/kg body weight. These substances also reduced healing times for ulcers induced in rats by chemical stress (acetic acid) or heat coagulation [42].

Wound healing effects

In vivo experiments
The wound healing activity of azulene has been demonstrated in studies on the thermally damaged rat tail [43] and of matricaria flower constituents in accelerated healing of experimental injuries [44].

Sedative effects

In vitro experiments
Apigenin competitively inhibited the binding of flunitrazepam to the central benzodiazepine receptor (K_i = 4 µM) but had no effect on muscarinic receptors, α_1-adrenoreceptors or on the binding of muscimol to $GABA_A$ receptors [45].

HPLC fractions of a methanolic extract of matricaria flower were able to displace flunitrazepam from its receptors in rat cerebellar membranes, the ligand Ro 5-4864 from 'peripheral' benzodiazepine receptors in rat adrenal gland membranes and muscimol from GABA receptors in rat cortical membranes. This last activity is mainly due to GABA present in the fractions [46].

Apigenin inhibited the binding of Ro 15-1788, a specific ligand for central benzodiazepine receptors with an IC_{50} of 0.25 mM. Apigenin also reduced GABA-activated Cl^- currents on cultured cerebellar granule cells dose-dependently by 15 ± 3% (0.1 µM apigenin), 24 ± 2% (1 µM apigenin) and 32 ± 4% (10 µM apigenin). This effect was blocked by co-application of Ro 15-1788 [47].

In vivo experiments
A sedative effect of matricaria flower was demonstrated through prolongation of hexobarbital-induced sleep, reduction of spontaneous mobility and reduction of explorative activity in mice [48,49].

Restriction stress-induced increases in plasma ACTH levels in normal and ovariectomized rats were decreased by administration of diazepam and inhalation of matricaria flower oil vapour. Inhaling the vapour induced greater decreases in plasma ACTH levels in ovariectomized rats than treatment with diazepam; this difference was not observed in normal rats. Furthermore, the inhalation of matricaria flower oil vapour induced a decrease in plasma ACTH level that was blocked by pretreatment with flumazenil, a potent and specific benzodiazepine receptor antagonist [50].

Apigenin (25 and 50 mg/kg) significantly reduced the time of latency ($p<0.05$) in the onset of picrotoxin-induced (6 and 8 mg/kg) convulsions [47]. Apigenin also reduced locomotor activity after intraperitoneal injection in rats (minimal effective dose: 25 mg/kg) but showed no anxiolytic, myorelaxant or anti-convulsant activity [51].

Antimicrobial effects
Matricaria flower oil exerted a bactericidal effect against Gram-positive bacteria and a fungicidal effect against *Candida albicans* at a concentration of 0.7% V/V. The oil was not active against Gram-negative bacteria even in concentrations as high as 8% V/V [52].

An infusion of matricaria flower, a hydroethanolic extract and pure herniarin exhibited antimicrobial activity against various bacteria and fungi in the presence of near UV light [53,54].

Pharmacological studies in humans

Anti-inflammatory effects
In a comparative open study involving 20 healthy volunteers with chemically-induced toxic dermatitis, the smoothing effect on the skin of an ointment containing matricaria flower extract was significantly superior ($p<0.01$) to that of 0.1% hydrocortisone acetate or the ointment base [55].

In an open study on 12 healthy subjects, a cream containing matricaria flower extract (20 mg/g) did not suppress UV-induced erythema but it reduced visual scores of skin redness in the adhesive tape stripping test ($p = 0.0625$) [56]. In an analogous study, the cream produced 69% of the effect of a hydrocortisone-

27-acetate ointment [57].

In a randomized, double-blind study, 25 healthy volunteers with UVB light-induced erythema were treated with various matricaria flower preparations, hydrocortisone cream or the respective vehicle. Ranking the preparations according to visual assessment scores and mean values from chromametry, a cream containing a hydroalcoholic extract of matricaria flower gave the best result [58].

Clinical studies

Anti-inflammatory effects

In a bilateral comparative study 161 patients with inflammatory dermatoses, who had been treated initially with 0.1% of diflucortolone valerate, were treated during maintenance therapy with a cream containing matricaria flower extract or one of three alternatives: 0.25% hydrocortisone, 0.75% fluocortin butyl ester or 5% bufexamac. The therapeutic results with the extract were equivalent to those of hydrocortisone and superior to those of fluocortin butyl ester and bufexamac [59].

In an open study involving 98 cancer patients, a matricaria flower extract preparation containing 50 mg of α-bisabolol and 150-300 mg of apigenin-7-glucoside per 100 g, applied three times daily, reduced oral mucositis caused by localized irradiation or systemic chemotherapy [60].

In a phase III double-blind, placebo-controlled study involving 164 patients, a mouth-wash containing matricaria flower extract did not decrease 5-fluorouracil-induced stomatitis [61].

In a randomized, partially double-blind, comparison study, 72 patients with medium-degree atopic eczema were treated with a cream containing a matricaria flower extract, or a 0.5% hydrocortisone cream or a placebo cream. After 2 weeks of treatment the matricaria cream proved superior to the hydrocortisone cream and marginally superior to the placebo cream with respect to the symptoms pruritus, erythema and desquamation [62].

Anti-inflammatory and antispasmodic effects

In an open multicentric study, 104 patients with gastrointestinal complaints such as gastritis, flatulence or minor spasms of the stomach were treated orally for 6 weeks with a matricaria flower extract preparation (standardized to 50 mg of α-bisabolol and 150-300 mg of apigenin-7-glucoside per 100 g) at a daily dose of 5 ml. Subjectively evaluated symptoms improved in all patients and disappeared in 44.2% of patients [63].

Wound healing effects

In an open study, 147 female patients episiotomized during childbirth were treated for 6 days with either an ointment containing matricaria flower extract or a 5% dexpanthenol cream. The healing effect of the two preparations was comparable [64].

In a randomized, double-blind, placebo-controlled study on 14 patients, weeping dermatoses following dermabrasion of tattoos were treated topically with a matricaria flower fluid extract preparation (standardized to 50 mg of α-bisabolol and 3 mg of chamazulene per 100 g). After 14 days the decrease in weeping wound area and the improvement in drying tendency were significant in the matricaria flower group ($p<0.05$) [15].

In a randomized, open, placebo-controlled study, 120 patients with second degree haemorrhoids were treated with rubber band ligature alone, rubber band ligature with anal dilator and vaseline, or rubber band ligature with anal dilator and an ointment containing matricaria flower extract. The last group showed the best results in amelioration of haemorrhage, itching, burning and oozing [29].

Pharmacokinetic properties

After cutaneous administration of [^{14}C](–)-α-bisabolol on mice, 82% of the radioactivity was found in the urine [65,66].

Apigenin and luteolin are also readily absorbed by the skin. Skin penetration studies using hydroethanolic solutions of apigenin and luteolin on the upper arms of 9 healthy female volunteers gave steady state fluxes of 10.31 ng/min/cm^2 and 6.11 ng/min/cm^2 respectively [67].

After oral administration of apigenin-7-glucoside to rats, free apigenin was detected in the urine [68].

After oral administration of 40 ml of a hydroethanolic matricaria flower extract (containing 225.5 mg of apigenin 7-glucoside, 22.5 mg of apigenin and 15.1 mg of herniarin per 100 ml) to a female volunteer, no flavones could be detected in blood plasma nor in 24-hour urine, while herniarin was found in both (maximum plasma concentration of ca. 35 ng/ml; 0.324 mg in 24-hour urine) [69].

In germ-free rats no hydrolysis of flavone glycosides could be observed; obviously intestinal microflora can effect the cleavage of the glycosidic bonds [68,70]. Furthermore, orally administered apigenin was detected in the blood serum of animals [71].

Preclinical safety data

The acute oral LD_{50} of matricaria flower oil in rats and the acute dermal LD_{50} in rabbits exceeded 5 g/kg. No irritant effects of the oil were observed after application

to the skin of nude mice [72].

In a 48-hour patch test in volunteers, matricaria oil neither caused skin irritation nor were there any discernible sensitization reactions or phototoxic effects. Matricaria oil has been granted GRAS status by FEMA and is approved by the FDA for use in food and cosmetics [72].

The acute oral toxicity of (–)-α-bisabolol in mice and rats was found to be very low, the LD_{50} being about 15 ml/kg. A six-week subacute toxicity study showed that the lowest toxic oral dose of (–)-α-bisabolol in rats and dogs was between 1 and 2 ml/kg. Oral doses up to 1 ml/kg of (–)-α-bisabolol produced no discernible effects on the prenatal development of rats or rabbits. No malformations were found at any of the dose levels tested [73].

The acute intraperitoneal LD_{50} of *cis*- and *trans*-en-yn-dicycloethers is 670 mg/kg [3]. In the Ames test, apigenin and an aqueous matricaria flower extract showed no mutagenic or toxic activity [74,75].

Allergenicity

Based on the fact that matricaria flower generally contains no, or only traces of, the sesquiterpene lactone anthecotulide and that millions of people come into contact with matricaria flower daily, allergic reactions due to matricaria flower can be considered to be extremely rare [32,76]. However, cross-reactions with other sesquiterpene lactone-containing plants are common [32]: 2 reports of a patient allergic to *Artemisia vulgaris* mention severe anaphylactic reactions following ingestion of matricaria flower infusions and after eye washing with similar infusions [77,78]; 18 of 24 patients with Compositae allergy were also allergic to an ether extract of matricaria flower [79]; 11 of 14 patients with Compositae allergy were allergic to an aqueous extract of matricaria flower [33]; 96 patients from 4800 showed contact hypersensitivity to an ethanolic matricaria flower extract [80]; 3 case reports mention an allergic reaction to matricaria flower and extract [81-83].

In a study of contact allergy performed with 540 type IV allergic patients, of whom some gave positive reactions to standard phytogenic allergens, none gave a positive reaction to an anthecotulide-free matricaria flower extract [84].

In a study with 830 patients with contact dermatitis only 1 patient gave a positive reaction to a matricaria flower extract and cream. Even a patient who was highly sensitive to *Anthemis cotula*, and another with oral allergy syndrome and hypersensitivity to many plants, tested negative [76].

These studies demonstrate the importance of using anthecotulide-free matricaria flower.

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MELILOTI HERBA

Melilot

DEFINITION

Melilot consists of the dried flowering tops of *Melilotus officinalis* Desr.

The material complies with the monograph of the Pharmacopée Française [1].

Fresh material may also be used provided that, when dried, it complies with the monograph of the Pharmacopée Française.

CONSTITUENTS

The main characteristic constituents are coumarin derivatives, especially melilotoside (*cis-o*-coumaric acid β-glucoside, approximately 0.5%) which lactonises to coumarin after hydrolysis [2,3,4]; free coumarin, 3,4-dihydrocoumarin (melilotin), scopoletin and umbelliferone are also present [5]. Other constituents include kaempferol and quercetin glycosides [5,6]; triterpene saponins based on soyasapogenols [6,7,8] and melilotigenin [9]; phenolic acids, principally melilotic acid (= *o*-dihydrocoumaric acid) and caffeic acid [10]; volatile compounds [11].

The pterocarpan medicarpin [12,13] and dicoumarol [5,13] are absent from properly dried melilot.

CLINICAL PARTICULARS

Therapeutic indications

Symptomatic treatment of problems related to varicose veins, such as painful and heavy legs, nocturnal cramps in the legs, itching and swelling [14,15].

Posology and method of administration

Dosage

Internal use

Drug or preparation corresponding to 3-30 mg of coumarin daily [16].

External use

Extracts in semi-solid preparations.

Method of administration

For oral administration and topical application.

Duration of administration

No restriction.

If symptoms persist or worsen, medical advice should

be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
Internal use may potentiate the activity of anti-coagulants [17].

Pregnancy and lactation
No abnormalities have been observed [18]. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In rare cases headaches have been reported after internal use [16].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments
Experiments on isolated segments of lymph vessels from guinea pigs demonstrated that a preparation containing melilot extract (coumarin 1.5 mg/ml) and rutin (15 mg/ml) had a marked myotropic action at an optimal dilution of $1:10^8$; pure coumarin had a similar effect at a dilution of $1:10^7$. Rhythm and tone were activated so that pulse rate, vascular amplitude and tone of lymphatic vessels increased considerably; there was also a rhythmifying effect on hypotonic vessels [19].

In vivo experiments
In experiments on carrageenan-induced rat paw oedema, intraperitoneal pre-treatment of the animals with coumarin (50 mg/kg body weight) isolated from melilot reduced the oedema by 42% after 4 hours and 33% after 6 hours compared to normal saline solution. The anti-oedematous effect was comparable to that obtained with flufenamic acid (1.5 mg/kg, administered intraperitoneally) [20].

A purified aqueous fraction from melilot containing 76-82% of polysaccharides was administered orally to 55 male rats and 120 female mice. Two doses were tested: 50 and 500 µg/kg body weight, once daily for 30 days. Rats treated with the extract showed increases in physical work capacity, their swimming time increasing by 38.5% ($p<0.001$) as compared to control (being longest on day 10 after the start of treatment), and in body weight throughout the 30-day treatment period, at the end of which they weighed 19.8% more than the control ($p<0.01$). In mice treated with the extract, the state of the peripheral blood and immuno-competent organs (spleen and thymus) was examined in detail. Both extract doses (50 and 500 µg/kg) led to decreases in spleen weight and significant rises in thymus weight and in erythrocyte, leukocyte, and particularly lymphocyte counts in the peripheral blood [21].

Clinical studies

Venous insufficiency
A comparative study was conducted in three groups (20 persons receiving 200 mg of a dry extract of melilot daily, 15 persons treated by ozonotherapy and 20 treated by the combined therapy). Administration of the melilot extract for 15 days significantly reduced some symptoms of chronic venous insufficiency, such as oedema ($p<0.0005$), nocturnal cramps ($p<0.05$) and heavy legs ($p<0.05$) [15].

A number of studies involving a total of 1818 patients have shown positive effects in cases of venous insufficiency and phlebitis, with a standardised preparation containing a melilot extract (0.05% of coumarin) in combination with rutin [18,22,23].

Lymphoedema
A group of 25 women with lymphoedema of the upper limbs due to axillary lymphadenectomy for breast cancer received 20 mg/day of a melilot extract containing 20% of coumarin for 12 weeks. A marked decrease in limb volume was observed after 6 weeks [24].

Another clinical study included 21 patients according to the following scheme: 4 patients as controls, 3 patients dropped out, 14 received a dry extract of melilot containing 8 mg of coumarin daily for 6 months. The extract was effective in reducing lymphoedema in 11 patients. The median reduction in upper arm circumference was about 5% compared to initial values [25].

Mastalgia
A study in 31 women showed that a melilot extract (dose not stated), taken daily for two periods of 2 months with an interval of 1 month, was effective in the treatment of cyclic mastalgias in 23 of the cases [26].

Pharmacokinetic properties.
In studies on human volunteers, coumarin admin-

istered orally as a dose of 0.857 mg/kg was rapidly absorbed, but only 2-6% reached systemic circulation in intact form. The rest of the dose appeared quantitatively in systemic circulation as the metabolites 7-hydroxycoumarin and its glucuronide, indicating an extensive first pass effect. The biological half-lives of coumarin and 7-hydroxycoumarin glucuronide were determined as 1.02 and 1.15 hours respectively. Approximately 90% of the dose was eliminated in the urine in the form of 7-hydroxycoumarin glucuronide [27].

It has been hypothesized that coumarin is the prodrug and 7-hydroxycoumarin (umbelliferone) the pharmacologically active moiety since the glucuronide, as a polar substance, should have no pharmacological activity [28].

Preclinical safety data

Acute toxicity
No data available for melilot. The intraperitoneal LD_{50} of a melilot/rutin preparation in mice was too high to be determined [29].
LD_{50} data for coumarin have been determined as follows:
In mice: oral, 196 mg/kg; intraperitoneal, 220 mg/kg; subcutaneous, 242 mg/kg.
In rats: oral, 293 mg/kg.
In guinea pigs: oral, 202 mg/kg [30].

Repeated dose toxicity
No data available.

Reproductive toxicity
The teratogenic effects of a combination of coumarin and rutin have been investigated in white New Zealand rabbits. Intravenous administration of either coumarin alone or a coumarin/rutin combination at 10 and 100 times the therapeutic dose during sensitive phases of foetal development did not result in any increase in malformation rates compared to controls. Treatment over a period of 13 days did not result in an increased number of resorptions or increased foetal mortality [31].

Clinical safety data

In a study in which 25 pregnant women were treated with a melilot/rutin preparation during their second and third trimesters, all the children were born normal [18].

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MELISSAE FOLIUM

Melissa Leaf

DEFINITION

Melissa leaf consists of the dried leaves of *Melissa officinalis* L. It contains not less than 4.0 per cent of total hydroxycinnamic derivatives expressed as rosmarinic acid ($C_{18}H_{16}O_8$; M_r 360.3), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [1].

Fresh material may be used provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

The main constituents are: essential oil (0.06-0.375% V/m) [2] containing monoterpenoid aldehydes, mainly geranial (citral a), neral (citral b) and citronellal [3-6]; flavonoids including glycosides of luteolin, quercetin, apigenin and kaempferol [7-9]; monoterpene glycosides [10,11]; phenylpropanoids, including hydroxycinnamic acid derivatives such as caffeic and chlorogenic acids, and in particular rosmarinic acid (up to 4%) [12-15]; triterpenes including ursolic and oleanolic acids [9,14].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Tenseness, restlessness and irritability; symptomatic treatment of digestive disorders such as minor spasms [16,17].

External use

Herpes labialis (cold sores) [18-21].

Posology and method of administration

Dosage

Internal use

2-3 g of the drug as an infusion, two to three times daily [16,17]. Tincture (1:5 in 45% ethanol), 2-6 ml three times daily [20]. Other equivalent preparations.

External use

Cream containing 1% of a lyophilised aqueous extract (70:1) two to four times daily [18-21].

Method of administration
For oral administration or topical application.

Duration of administration

Internal use
No restriction.

External use in *Herpes labialis*
From prodromal signs to a few days after the healing of lesions.

Contra-indications
None reported.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice the product should not be taken orally during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antispasmodic activity
Essential oil of melissa leaf showed spasmolytic activity when tested on isolated guinea pig ileum, rat duodenum and vas deferens, and on the jejunum and aorta of rabbits [22,23]. It also had relaxant effects on guinea pig tracheal muscle (EC_{50}: 22 mg/litre) and inhibited phasic contractions of an electrically-stimulated ileal myenteric plexus longitudinal muscle preparation (EC_{50}: 7.8 mg/litre) [24].

However, a hydroethanolic extract (1 part plant to 3.5 parts of ethanol 30% m/m) from melissa leaf at concentrations of 2.5 ml and 10 ml/litre did not show any significant antispasmodic activity when tested on acetylcholine- and histamine-induced contractions of guinea pig ileum [25].

Antiviral activity
Aqueous extracts exhibited antiviral activity against Newcastle disease virus, Semliki forest virus, influenza viruses, myxoviruses, vaccinia and *Herpes simplex* virus [26-29].

An aqueous extract from melissa leaf showed anti-HIV-1 activity (ED_{50}: 16 µg/ml). The active components in the extract were found to be polar substances. This extract also inhibited giant cell formation in co-culture of Molt-4 cells with and without HIV-1 infection and showed inhibitory activity against HIV-1 reverse transcriptase [30].

Anti-inflammatory activity
Rosmarinic acid has been shown to inhibit complement-dependent mechanisms of inflammatory reactions [31-33].

Antimicrobial activity
Melissa leaf essential oil was active against bacteria, filamentous fungi and yeasts [34].

Receptor-binding activity
Investigations were carried out to evaluate human CNS cholinergic receptor binding activity of an ethanolic extract of melissa leaf. The plant extract displaced [^{3}H]-(*N*)-nicotine and [^{3}H]-(*N*)-scopolamine from nicotinic and muscarinic receptors in homogenates of human cerebral cortical cell membranes (IC_{50} < 1 mg/ml). Choline, a weak nicotinic ligand (IC_{50}: 3×10^{-4} M), was found in melissa leaf extract at concentrations of 10^{-6} to 10^{-5} M. Melissa leaf extract had a high [^{3}H]-(N)-nicotine displacement value [35].

In a similar study in human occipital cortex tissue the IC_{50} for the displacement of [^{3}H]-(*N*)-nicotine and [^{3}H]-(*N*)-scopolamine from nicotinic and muscarinic receptors by a standardized extract (30% methanol; after evaporation the resulting soft extract was mixed with 10% of inert processing agents) were 11 mg/ml and 4 mg/ml respectively [36].

Antioxidant activity
Antioxidant and free radical scavenging properties have been reported for an aqueous extract [13,15,37-39]. 1,3-Benzodioxole isolated from a methanolic extract of melissa leaf has also been shown to have antioxidant activity [40].

In vivo experiments

Sedative effects
The sedative effect of a lyophilised hydroethanolic (30%) extract administered intraperitoneally to mice has been demonstrated by means of familiar (two compartment) and non-familiar (staircase) environment tests. The effect was dose-dependent up to 25 mg/kg body weight, the dose producing maximum

effects. Low doses (3-6 mg/kg) of the extract induced sleep in mice treated with an infra-hypnotic dose of pentobarbital and also prolonged pentobarbital-induced sleep. At high doses (400 mg/kg) a peripheral analgesic effect was noted in the acetic acid-induced writhing test, but no central analgesic effect was observed [41,42].

The essential oil administered intraperitoneally to mice had no effect in the staircase test nor was it active in prolonging pentobarbital-induced sleep [41]. When administered orally to mice it showed sedative and narcotic effects at doses of 3.16 mg/kg and higher [22].

Anti-inflammatory effects

Rosmarinic acid administered intravenously at 0.1-1 mg/kg inhibited cobra venom factor-induced rat paw oedema and exerted weak inhibition of carrageenan-induced paw oedema [32].

Other effects

An ethanolic liquid extract from melissa leaf was tested for its potential anti-ulcerogenic activity against indometacin-induced gastric ulcers in rats as well as for its antisecretory and cytoprotective activity. It showed dose-dependent anti-ulcerogenic activity at oral doses of 2.5-10 ml/kg associated with reduced acid output and increased mucin secretion, an increase in prostaglandin E_2 release and a decrease in leukotrienes. The effect on pepsin content was rather variable and did not seem to bear a relationship to the anti-ulcerogenic activity. The anti-ulcerogenic activity of the extract was also confirmed histologically. Cytoprotective effects of the extract could be partly due to its flavonoid content and to its free radical scavenging activity [43].

Rosmarinic acid inhibited passive cutaneous anaphylaxis in the rat with ID_{50} values of 1 mg/kg (intravenous) and 10 mg/kg (intramuscular) [32].

When applied topically (5% in vehicle) to rhesus monkeys, rosmarinic acid reduced both gingival and plaque indices over a 3-week study compared to placebo ($p<0.001$) [44].

Pharmacological studies in humans

A randomized, double-blind, placebo-controlled, crossover study was carried out in 20 healthy volunteers (mean age 19.2 years). The participants attended 4 days of treatment, receiving a single dose of either placebo or 300, 600 or 900 mg of a standardized melissa leaf extract (30% methanol; after evaporation the resulting soft extract was mixed with 10% of inert processing agents). Each treatment day was followed by a 7-day wash-out period. On each treatment day cognitive performance was assessed in a pre-dose testing session (baseline) and 1, 2.5, 4 and 6 hours after treatment using the Cognitive Drug Research computerised test battery and two serial subtraction tasks. Subjective mood was measured by Bond-Lader visual analogue scales. Significant improvement was observed for quality of attention at all times after a dose of 600 mg ($p = 0.0001$ to $p = 0.049$). Significant decreases in the quality of working memory and secondary memory were seen 2.5 and 4 hours after the higher doses ($p = 0.0005$ to $p = 0.05$). Reduction of working memory was more pronounced at 1 and 2.5 hours after the higher doses. Self-rated calmness was elevated significantly after 1 and 2.5 hours by the lowest dose ($p = 0.01$ to $p = 0.05$), while alertness was significantly reduced at all time points ($p = 0.001$ to $p = 0.05$) [36].

Clinical studies

A 4-week multicentre, double-blind, placebo-controlled study involved 72 patients of mean age 78.5 years with clinically significant agitation in the context of severe dementia. The patients were treated topically twice daily with a lotion containing 10% of melissa essential oil, providing a daily total of 200 mg of the oil ($n = 36$), or a placebo lotion ($n = 36$). Lotion was gently applied to the patient's face and both arms as an aromatherapy treatment. Changes in agitation were determined by the Cohen-Mansfield Agitation Inventory (CMAI) score. Improvements in the CMAI total score (35% reduction in the verum group and 11% in the placebo group) were significantly greater in the verum group ($p<0.0001$). A 30% improvement in CMAI score was attained by 21 subjects in the verum group compared to only 5 in the placebo group ($p<0.0001$). Quality of life indices measured by Dementia Care Mapping also improved significantly in the verum group; compared to the placebo group the percentage of time spent socially withdrawn was reduced ($p<0.005$) and time engaged in constructive activities increased ($p<0.001$) [45].

In a multicentre, open, controlled study involving 115 patients, a cream containing 1% of a lyophilised aqueous extract from melissa leaf (70:1) significantly reduced the healing time of cutaneous *Herpes simplex* lesions ($p<0.01$). It also significantly extended the intervals between recurrences of infection compared to other external virustatic preparations containing idoxuridine and tromantidine hydrochloride ($p<0.01$) [18,20]. These effects, particularly a significant reduction in the size of lesions within 5 days ($p = 0.01$), were confirmed in a multicentre, double-blind, placebo-controlled study on 116 patients [19,20].

A randomized, double-blind, placebo-controlled study was carried out using a cream containing 1% of a melissa leaf dry extract (70:1) standardized in terms of antiviral potency. Sixty-six patients with a history of recurrent *Herpes simplex labialis* (at least four episodes per year) were treated topically; 34 of them with verum cream and 32 with placebo. The cream was applied to the affected area 4 times daily over 5 days.

A symptom score (ranging between 0 and 9), derived by combination of the severity ratings for complaints, size of affected area and number of blisters on day 2 of therapy, was used as the primary target parameter. There was a significant difference ($p<0.05$) in scores for the primary target parameter between treatment groups: verum 4.03 ± 0.33 (3.0); placebo 4.94 ± 0.40 (5.0); values given are the mean ± SEM (median) of the symptom scores on day 2. The significant difference in symptom scores on the second day of treatment is of particular importance because the complaints in patients suffering from *Herpes labialis* are usually most intensive at that time [21].

Pharmacokinetic properties

No data available.

Preclinical safety data

Mutagenic activity

A tincture (ethanol 70%, 1:5) of melissa leaf gave negative results in the Ames test using *Salmonella typhimurium* TA 98 and TA 100 strains with or without metabolic activation [46]. No genotoxic effects from a 20% tincture of melissa leaf were detected in a somatic segregation assay using the diploid strain *Aspergillus nidulans* D-30 [47].

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MENTHAE PIPERITAE AETHEROLEUM

Peppermint Oil

DEFINITION

Peppermint oil is obtained by steam distillation from the fresh overground parts of the flowering plant of *Mentha* × *piperita* L.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

The main components of the oil are menthol, principally in the form of (–)-menthol (usually 35-55%) with smaller amounts of stereoisomers such as (+)-neomenthol (ca. 3%) and (+)-isomenthol (ca. 3%), and menthone (10-35%) [2-5].

Over 100 components have been identified in the oil including numerous other monoterpenes and small amounts of sesquiterpenes, notably viridiflorol (ca. 0.5%), which is characteristic of oil from *Mentha* × *piperita* [5].

To comply with the European Pharmacopoeia the oil must contain menthol (30-55%), menthone (14-32%), isomenthone (1.5-10%), menthyl acetate (2.8-10%), menthofuran (1-9%), cineole (3.5-14%), limonene (1-5%), not more than 4% of pulegone and not more than 1% of carvone, with a ratio of cineole content to limonene content greater than 2 [1].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Symptomatic treatment of digestive disorders, such as flatulence [6]; irritable bowel syndrome [6-10]; symptomatic treatment of coughs and colds [6,11-16].

External use

Relief of coughs and colds; symptomatic relief of rheumatic complaints [17]; tension-type headache [18]; pruritus, urticaria and pain in irritable skin conditions [19-22].

Posology and method of administration

Dosage

ADULTS

Internal use

For digestive disorders: 0.02-0.08 ml (1-4 drops) up to

three times daily [2,5,23] in dilute aqueous preparations (e.g. peppermint water or emulsion), or as drops on a lump of sugar [24].
For irritable bowel syndrome: 0.2-0.4 ml three times daily in enteric-coated capsules [2,6,7,25-30].

External use
As an inhalation (for coughs and colds): 3-4 drops added to hot water [5].
In dilute liquid or semi-solid preparations, as an anaesthetic or antipruritic (equivalent to 0.1-1.0% m/m menthol) or as a counter-irritant and analgesic (equivalent to 1.25-16% m/m menthol), rubbed on to the affected area [31].
Tension-type headache: as a 10% solution rubbed on to the skin of forehead and temples [18].

CHILDREN FROM 4-16 YEARS OF AGE
Internal use
For digestive disorders: proportion of adult dose according to body weight.

External use
Semi-solid preparations: *4-10 years*, 2-10%; *10-16 years*, 5-15% [32].
Hydroethanolic preparations: *4-10 years*, 2-4%; *10-16 years*, 3-6% [32].

Method of administration
For oral administration, local application or inhalation.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Contact sensitivity to peppermint oil or menthol [33-36].

Special warnings and special precautions for use
Direct application of peppermint oil preparations to the nasal area or chest of babies and small children must be avoided because of the risk of laryngeal and bronchial spasms [17,37-42].

Interaction with other medicaments and other forms of interaction
Patients with achlorhydria (caused, for example, by medication with H_2 receptor blockers) should use peppermint oil only in enteric-coated capsules [25].

Pregnancy and lactation
No data available. In accordance with general medical practice, peppermint oil should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects

Internal use
The use of non-enteric-coated peppermint oil preparations occasionally causes heartburn, especially in persons suffering from reflux oesophagitis [25,28, 30,43].

External use
Rare cases of skin irritation have been reported [44,45].
Inhalation of menthol can cause apnoea and laryngoconstriction in susceptible individuals [40].
Menthol can cause jaundice in newborn babies. In certain cases this has been related to glucose-6-phosphate dehydrogenase deficiency and other factors [46-48].

Overdose
Excessive inhalation of mentholated products has caused reversible, undesirable effects, such as nausea, anorexia, cardiac problems, ataxia and other CNS problems, probably due to the presence of volatile menthol [49-51].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Spasmolytic effects
Electrically-evoked contractions of isolated longitudinal smooth muscle from guinea pig ileum were inhibited by peppermint oil with IC_{50} values (concentrations which produced 50% inhibition) varying from 26 mg/litre [12,52] to 176 mg/litre [53], compared to 1.3-8 mg/litre for papaverine [12,52,53].
Chemically-evoked contractions of the guinea pig ileum were inhibited in the presence of increasing concentrations of peppermint oil (0.5×10^{-6} to 1×10^{-4}% V/V) [54].

Peppermint oil relaxed carbachol-contracted guinea pig taenia coli (IC_{50}: 22.1 µg/ml) and inhibited spontaneous activity in guinea pig colon (IC_{50}: 25.9 µg/ml) and rabbit jejunum (IC_{50}: 15.2 µg/ml) [55].

Peppermint oil also exhibited relaxant effects on tracheal smooth muscle of the guinea pig with an IC_{50} of 87 mg/litre [12]. The oil inhibited potential-dependent calcium currents in a concentration-dependent manner (IC_{50}: 15.2 µg/ml); this was recorded using the whole cell clamp configuration in rabbit jejunum smooth muscle cells [55].

More detailed studies on the mode of action of peppermint oil in the guinea-pig ileum revealed that the spasmolytic effect is post-synaptic and not atropine-like. Adrenoreceptors were also not involved. Using

a phosphodiesterase inhibitor, it was suggested that peppermint oil acts via a rise in intracellular cAMP, and not through cGMP, as shown by using a selective guanyl cyclase inhibitor. Peppermint oil was not considered to be acting as a potassium channel activator or a calcium channel blocker [56]. The latter is in contrast with other studies, which suggest that a reduction in calcium influx is involved. These differences might be due to different modes of action in different animal tissues (guinea pig taenia coli, rabbit jejunum) [54,55,57].

Other effects

Peppermint oil at concentrations of 20-50 µg/ml evoked ion permeability of heart cell membranes [57].

Menthol (10-30 µg/g tissue) antagonized contractions of the isolated frog rectum evoked by chemical and electrical stimulation. Vasodilatation was caused by the direct application of menthol to isolated ear vessels of rabbits [58].

Menthol has been reported to show antibacterial activity [20] and similarly peppermint oil showed antimicrobial activity [59]. Peppermint oil and 53 of its constituents were evaluated against *E. coli* in a preliminary screening test. It was found that peppermint oil and 3 of its constituents, menthol, menthone and neomenthol, had a bactericidal effect within 1 hour at concentrations of 400 µg/ml [60].

In vivo experiments

In experiments with anaesthetized guinea pigs, peppermint oil emulsified with tween 80 (0.1% in aqueous solution) caused resolution of a morphine-induced spasm of Oddi's sphincter. The oil administered intravenously at 1 mg/kg body weight partially unblocked Oddi's sphincter, which returned to normal in 17 minutes, whereas 3 mg/kg caused immediate total unblocking [61].

Peppermint oil appears to enhance production of bile [11]. In an anaesthetized, bile duct-cannulated dog, a peppermint leaf infusion (0.4 g/kg) strongly enhanced bile production [62]. Menthol also enhanced bile production: 0.06 g/kg in 1 dog [61] and 0.1-1.0 g/kg in rats [63].

Menthol dispersed in air, at concentrations of 140 ng/ml in cold air and 390 ng/ml in warm air, stimulated cold receptors in the respiratory tracts of 11 dogs and produced easier respiration [64]. Respiration was also facilitated when menthol was administered intravenously to 23 cats at 34.2 mg/kg [65].

Menthol inhibits hepatic HMGCoA reductase activity. Both menthol (468 mg/kg) and cineole (a minor constituent of peppermint oil; 262 mg/kg), separately administered orally to male rats, inhibited hepatic HMGCoA reductase activity by approximately 70% [66,67].

Pharmacological studies in humans

Peppermint oil (dose not stated) injected into the colon of 20 patients (through the biopsy channel of a colonoscope), relieved colonic spasms within 30 seconds [68], while 0.2 ml of peppermint oil in 50 ml of 0.9% sodium chloride with 0.01% polysorbate as suspending agent, injected into the colon of 6 subjects, relieved colonic spasms within 2 minutes, the effect lasting for about 12 minutes [69].

Two actions of peppermint oil on secretion in the respiratory tract have been reported: secretolytic in the bronchi [11,15,16,31] and decongestant in the nose [12].

Studies to assess the decongestant action of menthol have been carried out: on 62 volunteers with common cold, of whom 30 received a lozenge containing 11 mg of menthol [13]; on 29 healthy subjects breathing through an inhaler containing 125 mg of menthol dissolved in 1 ml of liquid paraffin [14]; and on 31 subjects receiving for 5 minutes menthol-containing air produced by passing the air through a flask containing approximately 1 g of menthol at 80°C [70,71]. All these studies showed that inhalation of menthol causes a subjective sensation of improved nasal air flow or 'easier breathing'. However, in subjects with common cold suffering from nasal congestion, inhalation of menthol produces no objective reduction in nasal airway resistance as measured by rhinomanometry [13,72].

The analgesic effect of peppermint oil (10% in ethanol) was investigated in 32 healthy subjects in a randomized, double-blind, placebo-controlled, four-arm crossover study. The peppermint oil preparation significantly reduced sensitivity to pain in experimentally-induced headache ($p<0.01$-0.001, depending on the method of induction used) when applied externally to the forehead and temples [73].

Menthol moderated oral sensations of warmth and coldness, as shown by an experiment on 31 young adults receiving a 0.02% aqueous solution of menthol in the mouth for 5 seconds [74].

Clinical studies

Irritable bowel syndrome

In an open, multicentre trial, 50 patients suffering from irritable bowel syndrome received an enteric-coated peppermint oil (0.2 ml) capsule 3 times per day, administered orally 30 minutes before a meal. Evaluation of all signs and symptoms confirmed a significant decrease in symptoms ($p<0.005$) after 4 weeks of treatment compared to initial values. No toxic effects were reported and undesirable side

effects were minimal and unimportant [7].

In two double-blind, crossover studies of irritable bowel syndrome with 16 and 29 patients respectively [25,26], enteric-coated peppermint oil (0.2 ml) capsules were compared with placebo. Three times daily the patients took 1 or 2 capsules depending on the severity of symptoms. Overall assessment of each treatment period showed that, compared to placebo, patients felt significantly better ($p<0.01$) while taking peppermint oil capsules and considered peppermint oil superior in relieving abdominal symptoms ($p<0.005$) [25].

In a double-blind, crossover study, 40 patients with irritable bowel syndrome were treated orally for 2 weeks with enteric-coated peppermint oil (0.2 ml) capsules or hyoscyamine (0.2 mg) or placebo. Treatment with peppermint oil tended to have a more pronounced effect on symptoms than hyoscyamine or placebo, but this was not statistically significant. These findings favour the short-term use of enteric-coated peppermint oil capsules as an antispasmodic in the treatment of irritable bowel syndrome [27].

In a double-blind clinical study 34 patients with irritable bowel syndrome, in whom pain was a prominent symptom, took 3 x 2 enteric-coated peppermint oil (0.2 ml) capsules or placebo daily. The patients' assessments at the end of 2 and 4 weeks of treatment showed no significant difference between peppermint oil and placebo in terms of overall symptoms [28].

In a randomized, double blind, placebo-controlled clinical study 110 outpatients (66 men and 44 women; 18-70 years of age) with symptoms of irritable bowel syndrome were assigned to oral treatment with 1 enteric-coated peppermint oil (0.2 ml) capsule or placebo 3-4 times daily, 15-30 minutes before meals, for a period of 1 month. The study was completed by 52 patients in the peppermint oil group and 49 in the placebo group. In the verum group, 79% of patients experienced a decrease in severity of abdominal pain (placebo group: 43%), 83% reported less abdominal distension (placebo group: 29%), 83% had reduced stool frequency (placebo group: 32%), 73% had fewer borborygmi (placebo group: 31%), and 79% less flatulence (placebo group: 22%). Improvements in all these symptoms were significantly better after peppermint oil than after placebo ($p<0.05$) [75].

In a randomized, double-blind, crossover study 18 patients with irritable bowel syndrome were treated orally with 3 enteric-coated peppermint oil (0.2 ml) capsules daily for 4 weeks and then changed to placebo for a further 4 weeks, or vice versa. Compared to placebo, peppermint oil produced a small but significant increase in frequency of defecation ($p<0.05$) but no significant change in scores for global severity of symptoms or scores for the specific symptoms of pain, bloating, urgent defecation and the sensation of incomplete evacuation [29].

A literature search revealed 8 randomized, controlled studies involving the use of enteric-coated peppermint oil capsules in irritable bowel syndrome and meeting pre-defined criteria for inclusion in a critical review; collectively they indicated that peppermint oil could be efficacious for symptomatic relief in irritable bowel syndrome. A meta-analysis of 5 of these studies (all double-blind, placebo-controlled) appeared to support this conclusion, showing a significant global improvement ($p<0.001$) in the symptoms of irritable bowel syndrome in patients treated with peppermint oil compared to placebo. However, in view of methodological flaws associated with most studies, no definitive judgement on efficacy could be given. The authors noted that well designed and carefully executed studies are needed to fully clarify the issue [76].

Tension-type headache

In a randomized, double-blind, placebo-controlled, crossover study involving 41 patients suffering from tension-type headaches, a total of 164 headache attacks were treated by oral medication (paracetamol 1000 mg or placebo) and simultaneously by cutaneous application of oil (a 10% solution of peppermint oil in ethanol, or a placebo with peppermint flavour). The oil was spread across the forehead and temples, and this was repeated after 15 and 30 minutes. Patients rated their pain intensities on a standardized category rating scale, making assessments after 15, 30, 45 and 60 minutes. The greatest effect was achieved with combined paracetamol/peppermint oil therapy ($p\leq0.001$ compared to placebo). Both peppermint oil and paracetamol also significantly reduced the intensity of clinical headache ($p<0.01$ compared to placebo) over the 1-hour observation period with no significant difference between these groups [18].

Pharmacokinetic properties

Pharmacokinetics in animals

After oral administration of menthol to rats in the high dosage range of 0.1-1.0 g/kg, menthol glucuronide was the main conjugate in urine, whereas the sulphate was predominant in bile [63].

Pharmacokinetics in humans

Menthol and other terpene constituents of peppermint oil are fat-soluble and therefore rapidly absorbed from the proximal small intestine when taken orally [30,77].

Urinary excretion of menthol (as the glucuronide) was studied in 13 healthy subjects after oral administration of a single dose of 0.6 ml of peppermint

oil, in the form of 3 enteric-coated peppermint oil (0.2 ml) capsules of one or the other of two different delayed-release preparations. With one of these formulations peak urinary excretion of menthol occurred 3 hours after administration; thereafter the levels rapidly decreased. The second preparation gave a peak urinary concentration of only about one-quarter of the first, but menthol excretion at this level was sustained over the period from 3 to 9 hours after administration [77].

In an earlier study, the pharmacokinetic profile of the second of the delayed-release preparations described above was compared with that of peppermint oil contained in soft gelatine capsules (which dissolve readily in the stomach).After oral administration to 6 healthy volunteers of a single dose providing 0.4 ml of peppermint oil, the total urinary excretion of menthol (as the glucuronide metabolite) was similar for both preparations, but peak menthol excretion levels were lower and excretion delayed with the delayed-release formulation [30].

Preclinical safety data

Peppermint oil was given orally (by gavage, diluted with soybean oil) to groups of 10 male and 10 female rats at 0, 10, 40 or 100 mg/kg body weight daily for 28 days. Histopathological changes, consisting of cyst-like spaces scattered in the white matter of the cerebellum, were seen at the higher dose levels of 40 and 100 mg/kg/day. There were no obvious signs of clinical symptoms due to the encephalopathy [78]. In a similar study in rats, but of longer duration, the same histopathological changes in the cerebellum were observed after oral administration of peppermint oil at 100 mg/kg/day for 90 days; in this study nephropathy was also observed in male rats at 100 mg/kg/day [79]. From these two studies the estimated no-effect-level of peppermint oil in rats was 10-40 mg/kg/day [78,79].

Oral administration of menthofuran to rats at a high dose level of 250 mg/kg/day for 3 days caused hepatotoxicity as indicated by a significant increase in serum glutamate pyruvate transaminase and decreases in glucose-6-phosphatase and aminopyrine *N*-demethylase activities. A decrease was also observed in the levels of liver microsomal cytochrome P-450, whereas cytochrome b_5 and NAD(P)H-cytochrome *c* reductase activities were unaffected [80].

Clinical safety data

A total of 323 patients and healthy volunteers have been included in 9 studies where efficacy and safety in the use of peppermint oil were investigated. Oral administration in capsules or direct injection into the colon varied from a single dose to 2 or 4 weeks of treatment with daily doses of 3-6 × 0.2 ml of the oil. In these studies there were no reports of toxicity and undesirable effects were minimal and unimportant; 1 patient developed a mild transient skin rash, and 1 experienced heartburn due to chewing the capsules [7,25-29,68,69,75].

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Reprinted with revisions and additions as: Göbel H, Schmidt G, Dworschak M, Stolze H, Heuss D. Essential plant oils and headache mechanisms. Phytomedicine 1995;2:93-102.
Also published with modifications as:
Göbel H, Schmidt G. Effekt von Pfefferminz- und Eukalyptusölpräparationen in experimentellen Kopfschmerzmodellen. Z Phytotherapie 1995;16:23-33.

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MENTHAE PIPERITAE FOLIUM

Peppermint Leaf

DEFINITION

Peppermint leaf consists of the whole or cut dried leaves of *Mentha* x *piperita* L. The whole drug contains not less than 12 ml/kg of essential oil. The cut drug contains not less than 9 ml/kg of essential oil.

The material complies with the monograph of the European Pharmacopoeia [1].

Fresh material may also be used, provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

The main active component is essential oil (1-3%), of which the principal constituent is usually menthol, in the form of (-)-menthol (usually 35-55 %) with smaller amounts of stereoisomers such as (+)-neomenthol (ca. 3%) and (+)-isomenthol (ca. 3%), together with menthone (10-35%), menthyl acetate, menthofuran, cineole, limonene and other monoterpenes [2,3]. Small amounts of sesquiterpenes occur in the oil, notably viridoflorol [3].

Various flavonoids are present including luteolin and its 7-glycoside [4], rutin, hesperidin [5], eriocitrin [6-8] and highly oxygenated flavones [4,9]. Other constituents include phenolic acids [8,10] and small amounts of triterpenes [11].

CLINICAL PARTICULARS

Therapeutic indications

Used in the symptomatic treatment of digestive disorders such as dyspepsia [5,12-16], flatulence [16,17] and gastritis [15], although no clinical data are available in support of these indications.

Posology and method of administration

Dosage

Adults: As an infusion, 1.5-3 g of the drug to 150 ml of water, three times daily [15,16]. Tincture (1:5, 45% ethanol), 2-3 ml, three times daily [16].
Elderly: Dose as for adults.
Children from 4 years of age, daily dose as infusions only: *4-10 years*, 3-5 g; *10-16 years*, 3-6 g [18].

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Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No harmful effects have been reported.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
The pharmacological actions of peppermint leaf are largely, but not exclusively, attributable to the essential oil; other components such as flavonoids also appear to play a role [15,16]. Pharmacodynamic data relating to the essential oil are given in the monograph on Peppermint oil.

In vitro experiments
A 30%-ethanolic extract from peppermint leaf exhibited antispasmodic activity at concentrations of 2.5 and 10.0 ml/litre, causing significant and dose-dependent increases in ED_{50} values for acetylcholine- and histamine-induced contractions of isolated guinea pig ileum ($p<0.01$ and $p<0.0005$ respectively for histamine-induced contractions), and a significant decrease in the maximum possible contractility ($p<0.05$ and $p<0.001$ respectively for histamine-induced contractions). The effect of the extract at 10.0 ml/litre corresponded to that of 1.6 µg of atropine per litre [13]. A similar peppermint leaf extract inhibited carbachol-induced contractions of isolated guinea pig ileum [14].

A total flavonoid fraction from peppermint leaf, dissolved in water so that 1 ml corresponded to about 0.5 g of dried leaf, inhibited barium chloride-induced contractions of isolated guinea pig ileum [19].

In vivo experiments

Choleretic effects
In experiments with cannulated dogs, peppermint tea (0.4 g/kg body weight) increased the secretion of bile [20]. Flavonoids, as well as the essential oil, contribute to this action [5,21,22].

Mixed flavonoids from peppermint leaf (optimum dose 2 mg/kg) showed choleretic activity in dogs [21]. Flavomentin, a flavonoid preparation from peppermint leaf, stimulated bile secretion and the synthesis of bile acids in dogs at doses of 0.5-6 mg/kg (optimum 2 mg/kg) [22].

In experiments with cannulated rats, intravenous injection of a peppermint tea at 0.5 ml/rat or a flavonoid preparation (corresponding to a dose of 3.3 g of peppermint leaf per kg body weight) proved effective in increasing the amount of bile acids [19].

Anti-ulcerogenic effect
Oral pre-treatment of rats with an ethanolic liquid extract from peppermint leaf at 2.5-10 ml/kg body weight gave dose-dependent protection against oral indometacin-induced gastric ulcers (80% protection at 10 ml/kg); this was confirmed histologically. The extract also had gastric antisecretory and cyto-protective effects; compared to rats treated intraperitoneally with indometacin (10 mg/kg), analysis of the gastric contents of animals pre-treated orally with the extract indicated reduced acid output, an increase in prostaglandin E_2 release and a decrease in leukotrienes (all $p<0.05$ at 2.5 ml/kg) [23].

Other effects
A dry 80%-ethanolic extract from peppermint leaf showed antinociceptive effects in mice. When administered orally at 200 mg/kg or 400 mg/kg, the extract significantly reduced acetic acid-induced writhing ($p<0.01$ and $p<0.001$ respectively). The response time of mice to thermal stimulation in the hot-plate test also increased significantly 45 and 60 minutes after intraperitoneal administration of the extract at 400 mg/kg ($p<0.01$ and $p<0.001$ respectively) [24].

The same extract showed anti-inflammatory activity against acute and chronic inflammation in rodents. After oral administration it reduced xylene-induced ear oedema in mice (acute model) by 49% at 200 mg/kg and 50% at 400 mg/kg (both $p<0.05$). After intraperitoneal administration in the cotton pellet granuloma test in rats (chronic model), only the higher dose of 400 mg/kg had a significant inhibitory effect ($p<0.01$) [24].

A dry aqueous extract of peppermint leaf, administered orally to mice as single doses of 300 or 1000 mg/kg body weight, caused weak sedative effects in several

tests: hexobarbital-induced sleep, exploratory behaviour, spontaneous motility and motor co-ordination. The same extract had a significant diuretic effect in mice at 100 and 300 mg/kg ($p<0.05$), but not at 1000 mg/kg [25].

Pharmacological studies in humans

The carminative action of peppermint leaf extracts is due to a reduction in tonus of the oesophageal sphincter, enabling release of entrapped air [17].

Pharmacokinetic properties

Pharmacokinetic data relating to the essential oil are given in the monograph on Peppermint oil.

Preclinical safety data

After oral administration of a dry peppermint leaf extract to 12 mice as a single dose at 4000 mg/kg body weight, none of the animals died and none showed macroscopic signs of toxicity over a 7-day period of observation [25].

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MYRRHA

Myrrh

DEFINITION

Myrrh consists of a gum-resin, hardened in air, obtained by incision or produced by spontaneous exudation from the stem and branches of *Commiphora molmol* Engler and/or other species of *Commiphora*.

The material complies with the monograph of the European Pharmacopoeia [1].

Species other than *Commiphora molmol* Engler [synonym: *C. myrrha* (Nees) Engler var. *molmol*] which may be acceptable sources of medicinal myrrh include *Commiphora abyssinica* (Berg) Engler and *C. schimperi* (Berg) Engler [2].

CONSTITUENTS

Myrrh can be separated into three components: volatile oil (2-10%), resin (25-40%) and gum (30-60%) [3].

The main constituents of the volatile oil are furanosesquiterpenes of various structural types including furanoeudesma-1,3-diene (principal component), furanoeudesma-1,4-diene-6-one, lindestrene, curzerenone, furanodiene, 2-methoxyfuranodiene and 4,5-dihydrofuranodiene-6-one, together with sesquiterpenes such as α-copaene, elemene and bourbonene [4-8].

Characteristic constituents of the resin are α-, β- and γ-commiphoric acids, α- and β-heerabomyrrhols, heeraboresene and burseracin [7,8]; also various terpenes [9] and a sesquiterpene lactone, commiferin [10].

The gum consists mainly of a proteoglycan in which chains of alternating galactose and 4-*O*-methylglucuronic acid, and separate chains of arabinose, are attached to the protein through hydroxyproline links [3,11,12].

CLINICAL PARTICULARS

Therapeutic indications

Topical treatment of gingivitis, stomatitis (aphthous ulcers), minor skin inflammations, minor wounds and abrasions; supportive treatment for pharyngitis, tonsillitis [8,13-17].

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Posology and method of administration

Dosage

Adults: As a gargle or mouthwash, 1-5 ml of tincture (1:5, ethanol 90% V/V) in a glass of water several times daily [8,13,17]. For use on skin, dab 2-3 times daily with diluted or undiluted tincture (1:5, ethanol 90% V/V) [8,13-16].

Elderly: as for adults.

Children: as for adults except using only diluted tincture on skin.

Method of administration
For topical application.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.
Because of the alcohol content, a transient burning sensation on the skin may be experienced depending on the level of dilution of the tincture.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Very rare cases of allergic contact dermatitis have been reported [18,19].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial and antifungal effects
Various sesquiterpene-containing fractions from myrrh inhibited *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans* with minimum inhibitory concentrations of 0.18-2.8 µg/ml [20].

In vivo experiments

Anti-inflammatory effects
In carrageenan-induced paw oedema and cotton pellet granuloma tests in rats a petroleum ether extract of myrrh (25:1), at an oral dose of 500 mg/kg body weight, exerted significant anti-inflammatory effects (Table 1) [21].

An ethanolic dry extract of myrrh (approximately 6:1), administered intraperitoneally to mice, exerted a significant anti-inflammatory effect ($p<0.05$) at 400 mg/kg body weight in the xylene-induced ear swelling model. The same extract significantly inhibited cotton pellet granuloma ($p<0.05$) in rats at an oral dose of 400 mg/kg [22].

Antipyretic effect
After oral administration to hyperpyretic mice of either an ethanolic or a petroleum ether extract of

TABLE 1

Treatment	Dose (mg/kg body weight)	Carrageenan-induced rat paw oedema		Cotton pellet-induced exudation	
		Mean increase in paw volume (ml ± SE)*	Per cent inhibition	Mean increase in weight of pellet (ml ± SE)*	Per cent inhibition
Control	Saline	0.86 ± 0.025	-	45.22 ± 2.18	-
Myrrh extract	500	0.33 ± 0.026	62.16	33.24 ± 3.65	26.49
Oxyphenbutazone	100	0.38 ± 0.017	55.32	Not tested	Not tested

*SE = Standard error of the mean

myrrh (25:1) at a dose of 500 mg/kg body weight a significant antipyretic effect ($p<0.001$) was demonstrated [21,23].

Stimulation of phagocytosis
Mice inoculated with *Escherichia coli* were treated intraperitoneally with either a dried ethanolic extract or the unsaponifiable fraction of myrrh, as solutions in aqueous ethanol (10% V/V) at 50 mg/kg (1 mg per 20 g animal). Both treatments stimulated phagocytosis in over 80% of the mice compared to controls [24].

Cytoprotective effect
Oral administration of myrrh to rats at 250, 500 and 1000 mg/kg body weight provided significant and dose-dependent protection to the gastric mucosa against the ulcerogenic effects of various necrotizing agents: 80% ethanol, 25% sodium chloride, 0.2 M sodium hydroxide, indometacin 30 mg/kg and combined ethanol 80%-indometacin 2.5 mg/kg ($p<0.05$ to $p<0.001$, depending on the dose). The same suspension significantly and dose-dependently protected against ethanol-induced depletion of gastric wall mucus ($p<0.05$ at 500 mg/kg; $p<0.001$ at 1000 mg/kg) [25].

Analgesic effects
In the hot plate test in mice a significant analgesic effect ($p<0.01$) was demonstrated after oral administration of myrrh at 1 mg/kg body weight [26].

Furanoeudesma-1,3-diene isolated from myrrh showed significant analgesic properties in mice when administered by intracerebroventricular injection at 1.25 mg/kg body weight ($p<0.01$) or orally at 50 mg/kg in the hot plate test, and also at 50 mg/kg in the writhing test. The analgesic effects were reversed by naloxone at 1 mg/kg, indicating an interaction with brain opioid receptors. This interaction was subsequently demonstrated *in vitro*; furanoeudesma-1,3-diene concentration-dependently displaced the specific binding of [^{3}H]diprenorphine to rat brain membrane [26,27].

An ethanolic dry extract of myrrh (approximately 6:1), administered orally to mice, exerted a significant and dose-dependent analgesic effect in the acetic acid-induced writhing test at 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) [22].

Antitumour and cytotoxic effects
After oral treatment of Ehrlich solid tumour (EST)-bearing mice with an aqueous suspension of myrrh at daily doses of 250 or 500 mg/kg body weight, the higher dose produced significant decreases ($p<0.05$) after 25 days and 50 days in tumour weight, in the viability of EST cells and in levels of DNA, RNA and protein in EST cells. The antitumour potential of myrrh was found to be comparable to that of the cytotoxic drug cyclophosphamide [28].

The antitumour activity of an aqueous suspension of myrrh, equivalent to that of cyclophosphamide, has also been demonstrated in Ehrlich ascites carcinoma (EAC) cell-bearing mice. At 500 mg/kg, significant reductions in the DNA ($p<0.05$), RNA ($p<0.01$) and protein ($p<0.01$) contents of EAC cells, and in their viability ($p<0.05$), were observed together with an increased survival rate of the animals [29].

Hypoglycaemic effects
Intragastric treatment of normal and diabetic rats with a 5% m/V aqueous extract of myrrh (extracted with boiling water then filtered) daily for one week at 10 ml/kg body weight lowered fasting blood glucose levels in both groups and, in the oral glucose tolerance test, significantly increased glucose tolerance in both normal ($p<0.02$) and diabetic animals ($p<0.05$) [30].

Oral administration of two fractions (200-250 mg/kg body weight) and two pure furanosesquiterpenes (150-175 mg/kg) from myrrh (*C. myrrha*) to obese diabetic mice produced significant reductions in blood glucose at 27 hours post-dose ($p<0.005$ in all cases). One active fraction at 200 mg/kg reduced blood glucose by 50% ($p<0.0001$), compared to a 41% reduction with the oral antidiabetic metformin at 250 mg/kg [31].

Antithrombotic activity
Powdered myrrh (*C. molmol*), administered orally at 100 mg/kg body weight, provided 86% protection against experimental thrombosis in mice ($p<0.05$), comparable to the effect of acetylsalicylic acid at 20 mg/kg [32].

Local anaesthetic activity
A fraction from myrrh (*C. molmol*) composed of furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, administered as eye drops at a concentration of 280 µg/ml into the conjunctival sac of rabbits, had a strong local anaesthetic effect ($p<0.01$ compared to the vehicle as control) of about half that of procaine at 100 µg/ml [20].

Clinical studies

Anthelmintic effects
Oral treatment of 204 patients suffering from schistosomiasis with myrrh at 10 mg/kg body weight/day for 3 days in an open study produced a cure rate of 91.7%. Non-responding patients treated for 6 further days with the same dose gave a cure rate of 76.5%, increasing the overall rate to 98%. 20 patients provided biopsy specimens 6 months after treatment and none of them showed living ova [33].

In a preliminary open study 7 patients with fascioliasis (infection with parasitic liver flukes) were treated orally with a preparation consisting of 8 parts of myrrh resin and 3.5 parts of myrrh volatile oil at 12 mg/kg

body weight/day for 6 days. The therapy proved to be effective, with pronounced improvement in the general condition of the patients and amelioration of all symptoms and signs. By the end of treatment a dramatic drop in the egg count was observed and eggs were no longer detectable in the faeces after 3 weeks or after a follow-up period of 3 months. High eosinophilic counts, elevated liver enzymes and *Fasciola* antibody titres returned to nearly normal [34].

Pharmacokinetic properties
No data available.

Preclinical safety data
The acute oral LD_{50} of myrrh oil has been determined as 1.65 g/kg [35].

Myrrh and the volatile oil of myrrh are reported to be non-irritating, non-sensitizing and non-phototoxic when applied to animal or human skin [16,35].

Oral toxicity studies of myrrh (*C. molmol*) were carried out in mice using acute doses of 0.5, 1.0 and 3.0 g/kg body weight and chronic doses of 100 mg/kg/day for 90 days. Compared to controls, no significant differences in mortality, weight gain or biochemical parameters were observed after acute or chronic treatment. After chronic treatment there were significant increases in weight of testes and seminal vesicles ($p<0.05$) and of caudae epididymis ($p<0.01$), and a significant increase in red blood cell count and haemoglobin ($p<0.05$). The toxicity studies supported the safe medicinal use of myrrh [36]

Genotoxicity and cytotoxicity
Myrrh administered orally to normal mice for 7 days at 125-500 mg/kg body weight/day as an aqueous suspension showed no mutagenicity in the micronucleus test. It caused a significant, dose-dependent reduction in the RNA content of hepatic cells ($p<0.01$ at 250 mg/kg), but not the DNA or protein content, and a highly significant, dose-dependent, mitosis-depressant effect in femoral cells ($p<0.001$) [29,37].

Clinical safety data
Myrrh was well tolerated with only mild and transient side effects when administered orally to 204 patients with schistosomiasis at 10 mg/kg body weight/day for 3-9 days [33]. No signs of toxicity or adverse effects were observed from treatment of 7 patients with fascioliasis with myrrh resin/volatile oil at 12 mg/kg body weight for 6 days [34].

Ethnopharmacological evidence [16] suggests that myrrh has been extensively used both internally and externally without serious adverse effects.

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MYRTILLI FRUCTUS

Bilberry Fruit

DEFINITIONS

Dried bilberry fruit consists of the dried ripe fruit of *Vaccinium myrtillus* L. It contains not less than 1.0 percent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) and calculated with reference to the dried drug.

Fresh bilberry fruit consists of the fresh or frozen ripe fruit of *Vaccinium myrtillus* L. It contains not less than 0.30 per cent of anthocyanins, expressed as cyanidin-3-glucoside chloride (chrysanthemin $C_{21}H_{21}ClO_{11}$; M_r 485.5) and calculated with reference to the dried drug.

The materials comply with the respective monographs of the European Pharmacopoeia [1,2].

CONSTITUENTS

The main characteristic constituents are anthocyanins (anthocyanosides; 0.5% in dried bilberry fruit) [3]. Other constituents include tannins, hydroxycinnamic and hydroxy-benzoic acids, flavonol glycosides, flavan-3-ols, iridoids, terpenes, pectins and organic plant acids [3-7].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Extracts of bilberry fruit enriched in anthocyanins: symptomatic treatment of problems related to varicose veins, such as painful and heavy legs [8-17].
Dried bilberry fruit: supportive treatment of acute, non-specific diarrhoea [18,19].

External use

Topical treatment of mild inflammation of the mucous membranes of the mouth and throat [18].

Posology and method of administration

Dosage

Internal use

Standardized extracts of bilberry fruit containing 36% of anthocyanins: 320-480 mg/day [20-29]; equivalent preparations.
Dried bilberry fruit: 20-60 g daily [18,19].

External use

A 10% decoction of dried bilberry fruit [18].

Method of administration
For oral administration or local application.

Duration of administration
No restriction.
If diarrhoea persists for more than 3-4 days medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Anthocyanins are well tolerated in pregnancy; they do not induce side-effects in the mother or offspring [17].

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

In the following text, unless stated otherwise, "standardized extract" of bilberry fruit refers to an extract containing 36% of anthocyanins.

Pharmacodynamic properties

In vitro experiments

Antioxidant activity
Anthocyanin-rich extract of bilberry fruit is reported to be a potent scavenger of free radicals, behaving both as a scavenger against superoxide anion [30-33] and as an inhibitor of lipid peroxidation induced by adenosine diphosphate (ADP)/Fe^{2+} and ascorbate in rat liver microsomes [31-33]. The radical scavenging properties have also been verified for individual anthocyanins [34]. Furthermore, an anthocyanin-rich extract inhibited the K^+ loss induced by free radicals in human erythrocytes as well as the cellular damage caused by oxidant compounds such as daunomycin and paraquat [35,36].

More recently an aqueous extract of bilberry fruit was shown to have a potent protective action on human low density lipoprotein (LDL) during copper-mediated oxidation [37], and a standardized extract containing 37% of anthocyanins prevented photo-induced oxidation of human LDL and fragmentation of apoprotein [38].

Platelet aggregation
A standardized extract of bilberry fruit showed activity against aggregation induced by ADP, collagen and sodium arachidonate in rabbit platelet-rich plasma [39]. This was confirmed *ex vivo* on ADP- and collagen-induced aggregation of platelets obtained from the blood of 30 healthy volunteers given 480 mg/day of the standardized extract orally for 30-60 days [40]. An anthocyanin extract of bilberry fruit also inhibited *in vitro* platelet aggregation induced by ADP or adrenaline in human plasma [41].

Effect on vascular tissues
Anthocyanin-rich extract of bilberry fruit is able to inhibit proteolytic enzymes such as elastase [42] and to interact with collagen metabolism by cross-linking collagen fibres, making them more resistant to collagenase action [43] and reducing the biosynthesis of polymeric collagen [44].

An anthocyanin-rich standardized extract (corresponding to 25% of anthocyanidins) had a slightly relaxing effect on various isolated vascular smooth muscle preparations and reduced the response to contraction inducers such as serotonin and barium [45-48].

In vivo experiments

Antioxidant activity
Oral pre-treatment of mice with 250 or 500 mg/kg of an anthocyanin-rich extract from bilberry fruit inhibited liver lipid peroxidation stimulated by a mixture of $FeCl_2$, ascorbic acid and ADP. The malonaldeyde content in the liver was significantly reduced ($p<0.05$) [33].

Vasoprotective activity
Anthocyanin-rich extracts of bilberry fruit exert modulating effects on capillary resistance and permeability, as demonstrated in various experimental models [20-22,49,50]. For example, an extract administered orally to rabbits at 200-400 mg/kg protected against increased capillary permeability induced by topical application of chloroform [20]. When administered orally to rats at 100-200 mg/kg, or intraperitoneally or intramuscularly at 25-100 mg/kg, the extract gave protection from capillary lesions induced by intradermal injection of bradykinin [20]. In a more recent study, the same extract was found to antagonise damage induced by ischaemia-reperfusion in the hamster cheek-pouch microcirculation model after oral administration at 100 mg/kg for 4 weeks [23].

Ophthalmic activity
An anthocyanin-rich extract of bilberry fruit, administered intravenously to rabbits at 3.2 mg/kg, reduced the permeability of vessels of the ciliary body, which had been increased by paracentesis [24].

Furthermore, a mixture of anthocyanins administered intravenously to rabbits at 160 mg/kg promoted dark adaptation after dazzling [25]. It was suggested that this improvement in visual function was probably due to an increase in the regeneration rate of rhodopsin [26].

Anti-inflammatory activity
The vasoprotective effect could also be responsible for anti-inflammatory activity exhibited by anthocyanins (50-500 mg/kg, given orally) against rat paw oedema induced by irritant agents such as carrageenan, histamine or hyaluronidase [20,27].

Wound healing and antiulcer activity
Topical application of 5-10 mg of an anthocyanin-rich extract of bilberry fruit accelerated the healing of experimental wounds in rats [28]. In another experimental model, healing delayed by prednisone, the same extract applied topically at concentrations of 0.5-2% for 3 days to skin wounds in rats promoted healing activity in comparison with prednisone-treated controls [29].

A standardized extract (corresponding to 25% of anthocyanidins), administered orally to rats at doses ranging from 25 to 200 mg/kg, showed antiulcer activity against acute gastric ulcers induced by pyloric ligation, non-steroidal anti-inflammatory drugs (NSAIDs) and reserpine [29].

Antiatherogenic activity
An anthocyanin-rich extract of bilberry fruit, administered intraperitoneally at 100 mg/kg for 45 days to rabbits fed on a cholesterol-rich diet, reduced the proliferation of intima and calcium and lipid deposition on the aortic wall [51].

Clinical studies

Peripheral vascular diseases
In 47 patients with lower limb varicose syndrome, oral treatment with bilberry fruit extract at 480 mg/day for 30 days led to improvements in objective symptoms such as limb oedema and dyschromic skin phenomena, and subjective symptoms such as heaviness, paraesthesia and pain [8].

Improvements in vessel fragility and objective symptoms after administration of bilberry fruit extract at 480 mg/day for 30-180 days have been confirmed in trials performed on 22 diabetic and dyslipidaemic patients [9], on 97 patients with complaints induced by stasis of the lower extremities such as prevaricose syndrome, essential varices and post-phlebitic syndrome [10], and on 42 patients with severe arteriosclerotic vascular disease of the lower limbs [11].

In a double-blind, placebo-controlled study performed on 47 patients with peripheral vascular disorders of various origins, a standardized extract of bilberry fruit (480 mg/day for 30 days) reduced subjective symptoms such as paraesthesia, pain and heaviness, and improved oedema and mobility of finger joints [12].

The efficacy of a standardized extract of bilberry fruit has been evaluated in two clinical studies, each in 15 patients with venous insufficiency who were treated orally with 480 mg/day for 2-4 months. Significant improvements in plethysmographic ($p<0.05$) and duoregional rheographic ($p<0.001$) observations were reported [13,14].

In 24 patients with chronic venous insufficiency, oral administration of 480 mg of an anthocyanin-rich extract of bilberry fruit daily for 60 days induced a decrease in the total time of drainage after reactive hyperaemia evaluated by the strain gauge technique [15].

The efficacy of the same extract (480 mg/day for 30 days) was further demonstrated in a single-blind, placebo-controlled study carried out in 60 patients with different stages of venous insufficiency; this study showed significant activity of the extract ($p<0.01$ to $p<0.001$) on subjective parameters, namely feeling of pressure in the lower limbs and muscle cramps as well as oedema and leg and ankle girth [16].

Significant improvements in subjective symptoms ($p<0.01$) and reductions in oedema and capillary fragility were observed in 54 cases of phlebopathies induced by stasis during pregnancy after oral administration of 320 mg of extract daily for 60-90 days [17].

Ophthalmic disorders
Daily treatment of 14 patients suffering from tapetoretinal degeneration with 3 × 150 mg of a bilberry fruit extract resulted in an improvement in light sensitivity of the retina starting from the second day of treatment and remaining almost constant during the 3-month treatment period [52].

The efficacy of a standardized extract (320 mg/day) has been evaluated in 40 patients with refractory defects. Accurate ocular and electrofunctional examinations showed a significant increase in the flash electroretinogram amplitude in medium ($p = 0.002$) and high myopia ($p = 0.008$), indicating an improvement in retinal sensitivity [53]. In another study, daily administration of 320 mg of the extract for 3 months to 26 myopic patients resulted in improvement of electrophysiological functions [54].

In a double-blind, placebo-controlled study involving 40 healthy subjects the activity of an anthocyanin-rich extract, administered orally at 240 mg daily for 3 months, was evaluated from pupillary movements through examination of the direct photomotor reflex. The study demonstrated a more efficient pupillary photomotor response after administration of the extract compared to placebo [55].

Oral administration of a standardized extract of bilberry fruit at 480 mg/day for 180 days to 10 patients with type II diabetes mellitus and non-proliferative retinopathy resulted in improvement of the diabetic retinopathy, with marked reduction or disappearance of retinal haemorrhages [56].

In a double blind, placebo-controlled study, 40 patients with diabetic or hypertensive retinopathy were treated with 320 mg of a standardized extract of bilberry fruit or placebo daily for 30 days. An improvement in ophthalmoscopic and angiographic patterns was observed in 77-90% of the verum patients [57].

In another placebo-controlled study, involving 40 patients with diabetic retinopathy at a relatively early phase, a standardized extract of bilberry fruit administered at 320 mg/day for 12 months promoted the regression of hard exudates, which is considered a reliable index of altered permeability [58].

Pharmacokinetic properties

After oral administration to rats of a single dose of 400 mg/kg, a standardized extract of bilberry fruit was rapidly absorbed from the gastrointestinal tract with a C_{max} of 2.47 µg/ml and at a T_{max} of 15 min [59].

After intravenous or intraperitoneal administration of an anthocyanin-rich extract (equivalent to 25% of anthocyanidins) to rats at doses of 20-40 mg/kg or 25 mg/kg, respectively, anthocyanins were rapidly distributed to the tissues. Elimination occurred mainly in the urine (25-30% of the dose within 24 hours after administration) and to a lesser extent (15-20%) in bile [60].

Preclinical safety data

Acute toxicity

Intraperitoneal and intravenous LD_{50} values of an anthocyanin-rich extract from bilberry fruit containing about 70% of anthocyanins were determined as 4.11 g/kg and 0.84 g/kg in the mouse, and 2.35 g/kg and 0.24 g/kg in the rat, respectively. No deaths were observed following oral doses of up to 25 g/kg in the mouse and 20 g/kg in the rat [50].

Chronic toxicity

Treatment of rats for 90 days with the same extract at a daily dose corresponding to approximatively five times the human clinical dose (i.e. 600 mg/day) did not produce any toxic effects [50].

Mutagenicity and carcinogenicity

No data are available.

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ONONIDIS RADIX

Restharrow Root

DEFINITION

Restharrow root consists of the dried roots of *Ononis spinosa* L.

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

Isoflavones including trifolirhizin [2,3], formononetin [3-5] together with its 7-O-glucoside-6″-malonate [4] and 7-O-glucoside (= ononin) [3,4], biochanin A 7-O-glucoside [4,5], medicarpin [6] and related compounds [3-5]; triterpenes, notably α-onocerin [3,7-9]; phytosterols, especially β-sitosterol [3,7]; phenolic acids [10]; tannins [11]; minerals [12] and about 0.02% of essential oil [13-15] containing mainly *trans*-anethole, carvone, menthol [13] and aromatic hydrocarbons [15]. The presence of flavonols such as rutin and kaempferol [5] has not been confirmed [16,17].

CLINICAL PARTICULARS

Therapeutic indications
Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in treatment of bacterial infections of the urinary tract [12,18-21].

Posology and method of administration

Dosage

Adults: An infusion of 2-3 g of dried material two to three times per day; equivalent preparations [22].

Method of administration
For oral administration. To prepare an infusion, boiling water is poured over the material and the mixture strained after 20-30 minutes.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Diuretic activity

In vivo experiments
In studies on 250 g rats, four preparations of restharrow (presumed to be from the root) were administered intragastrically to different groups of 4 rats, each dose being equivalent to 0.3 g of root and including 20 ml of water. The controls were water or theophylline (5 mg/kg), administered to the same rats several days later. The volumes of urine collected from the rats over a 5-hour period following administration of the respective preparations were: dried methanolic extract (rich in flavonoids) 19.9 ml, ash (rich in minerals, especially potassium) 18.7 ml, a mixture of methanolic extract and ash 20.9 ml, and aqueous infusion 21.4 ml, compared to 15.1 ml for water and 17.9 ml for theophylline. Corresponding amounts of sodium, determined by atomic absorption spectrophotometry in the urine collected over 5 hours, were 20, 32, 21 and 24 mg, compared to 6 and 16 mg with water and theophylline respectively. The potassium figures were 95, 79, 66 and 62 mg, compared to 44 and 61 mg with water and theophylline. These results demonstrated moderate diuretic ($p<0.001$) and saluretic activity, particularly natriuretic, of the preparations, in all cases higher than that of theophylline at 5 mg/kg. It was concluded that the diuretic activity of restharrow root was caused by its content of potassium salts and flavonoid glycosides [23].

An ethanolic extract (not defined) at a dose corresponding to 2 g of drug per kg body weight significantly increased urinary volume by 103% ($p<0.05$) compared to saline. No influence was observed on sodium or potassium elimination. Intraperitoneal administration did not show any diuretic effect at doses of drug up to 500 mg/kg [24].

Older studies gave the following results: an infusion from restharrow root administered orally to rabbits increased the urinary output by approx. 26% [25]; in mice an increase of urinary output and chloride excretion was demonstrated [25], while in rats the excretion of urea and chloride increased after oral administration [26].

After oral administration to rats, infusions of the root caused slight diuresis (average of 12%) and decoctions an antidiuretic effect of 7-20%. In these studies the aqueous residue after steam distillation showed an antidiuretic effect of 7-16% depending on the duration of distillation, whereas 0.5-1.0 ml of the essential oil obtained by steam distillation (2-4 hours) produced a diuretic effect [27]. The author [27] concluded that only the essential oil of restharrow root exhibits diuretic activity, a point which has been hotly disputed in the past [28-30].

Other effects

In vitro experiments
A methanolic restharrow root extract (6:1) was found to inhibit 5-lipoxygenase selectively with an IC_{50} of 7.8 µg/ml. The pterocarpan medicarpin, isolated from the extract, inhibited leukotriene B_4 formation with an IC_{50} of 6.7 µM [6].

Extracts of restharrow root have been shown to have antifungal activity [31].

In vivo experiments
No analgesic effects were observed in the hot plate test in mice after oral or intraperitoneal administration of an ethanolic restharrow root extract (not further defined). In the phenylquinone writhing test in mice, the extract reduced reaction to pain by up to 80% at doses of 100 and 500 mg per kg body weight after intraperitoneal administration, while no effect was observed after oral administration [24].

In the carrageenan-induced rat paw oedema test, the same extract significantly reduced oedema by a maximum of 46% after 3 hours ($p<0.05$) at an intraperitoneal dose corresponding to 500 mg of restharrow root per kg body weight, while no significant effects were observed at 100 mg/kg. Information on the controls was not stated [24].

Pharmacokinetic properties
No data available.

Preclinical safety data
An ethanolic extract (not further defined), administered orally or intraperitoneally at a daily dose corresponding to 2 g of drug per kg body weight for 14 days, did not cause any visible toxic effects [24].

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ORTHOSIPHONIS FOLIUM

Java Tea

DEFINITION

Java tea consists of the fragmented, dried leaves and tops of stems of *Orthosiphon stamineus* Benth. (*O. aristatus* Miq.; *O. spicatus* Bak.).

The material complies with the monograph of the European Pharmacopoeia [1].

CONSTITUENTS

Up to 12% of minerals with a high proportion of potassium [2-10], approx. 0.2% of lipophilic flavones including sinensetin and isosinensetin [2,3,6,7,11-18], flavonol glycosides [15,16], rosmarinic acid (0.1-0.5%) [3,16,18-20] and other caffeic acid depsides [15,16], inositol [8], phytosterols such as β-sitosterol [2] and up to 0.7% of essential oil [2,4,6,7,9,10,21]; pimarane, isopimarane and staminane diterpenes [18,22-29], triterpenes [2,6,7,9,16,29,30] and chromenes such as methylripariochromene A [28,31,32].

CLINICAL PARTICULARS

Therapeutic indications
Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in the treatment of bacterial infections of the urinary tract [2,7,16,33-35].

Posology and method of administration

Dosage
Adults: An infusion of 2-3 g of dried material in 150 ml of water two to three times per day; equivalent preparations [3,5,7,36].

Method of administration
For oral administration.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
Java tea should not be used in patients with oedema due to impaired heart and kidney function.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial activity
Bacteriostatic [8, 37] and fungistatic [38] activity has been demonstrated, and the bacteriostatic activity has been attributed to caffeic acid derivatives, particularly rosmarinic acid [16].

Effects on lipoxygenase activity
Various isolated lipophilic flavones inhibited 15-lipoxygenase from soybeans (used as a model for mammalian 15-lipoxygenase) [39]. In another study, flavonoids isolated from Java tea prevented inactivation of soybean 15-lipoxygenase, caused by bubbling air through the enzyme solution. The compounds with the strongest enzyme-stabilizing effects, 5,7,4′-trimethylapigenin, eupatorin and 5,7,3′,4′-tetramethylluteolin, gave 50% protection at concentrations of 2.0, 2.4 and 4.3 µM respectively. However, none of the flavonoids were efficient as scavengers of the diphenylpicrylhydrazyl (DPPH) radical [40].

In vivo experiments

Diuretic activity
A 5% infusion of Java tea administered intravenously to rabbits had a diuretic effect [9]. This was also observed after subcutaneous injection of an aqueous extract into rabbits and dogs [41]. The volume of urine and excretion of electrolytes (K^+, Na^+, Cl^-) were increased by intravenous infusion into dogs of a 50% ethanolic extract at 18.8 mg/kg/min [42]. Oral administration to rats of a lyophilized aqueous extract at 750 mg/kg body weight enhanced ion excretion (K^+, Na^+, Cl^-) to a level comparable to that obtained with furosemide at 100 mg/kg, but no aquaretic effect was observed [43].

Although it is not yet clear which are the active compounds [16], the diuretic effect could be partially due to the potassium content of Java tea [8,43] as well as to the flavones sinensetin and 3′-hydroxy-5,6,7,4′-tetramethoxyflavone, which exhibited diuretic activity in rats after intravenous administration of 10 mg/kg body weight [44].

Aqueous (5:1) and ethanolic (7:1, ethanol 70°) spray-dried extracts of Java tea were administered intragastrically to male rats as single doses at two levels: the aqueous extract at 18.0 mg/kg and 180 mg/kg, the ethanolic extract at 13.5 mg/kg and 135 mg/kg. Each extract was given to 5 rats, a further 5 rats receiving water as controls, and urine was collected over a period of 6 hours. After all doses the urine volume was between 2.99 and 3.36 ml per 100 g of rat, significantly higher ($p \leq 0.05$) than that of controls (2.15 ml). With both doses of the aqueous extract, a significant increase ($p = 0.009$) in sodium elimination was observed (0.1-0.12 mEq per 100 g of rat) compared to controls (0.05 mEq). The higher doses of aqueous and ethanolic extracts significantly ($p = 0.009$ and $p = 0.02$ respectively) increased chloride elimination (0.11 and 0.09 mEq per 100 g of rat) compared to controls (0.07 mEq). No significant changes were observed in elimination of potassium or urea [45].

A hydroethanolic extract was administered intraperitoneally to 13 male rats as a single dose of 50 mg/kg body weight; a control group of 28 rats received hypotonic saline solution. Another group of 10 rats received hydrochlorothiazide at 10 mg/kg body weight. After 8 hours the urine volume from rats treated with the extract was significantly higher ($p<0.001$) than that of the controls and was comparable to the volume after hydrochlorothiazide treatment [46].

Methylripariochromene A isolated from Java tea was suspended in 0.5% Tween 80 and administered orally to 5 rats at three dose levels (25, 50 and 100 mg/kg body weight) followed by oral administration of saline at 20 ml/kg. The highest dose produced a significant increase ($p<0.01$) in urinary volume over a period of 3 hours, comparable to that of hydrochlorothiazide at 25 mg/kg. Excretion of K^+, Na^+ and Cl^- increased significantly at 100 mg/kg body weight compared to controls ($p<0.05$, $p<0.01$ and $p<0.01$ respectively), but less than with hydrochlorothiazide [28,47].

Anti-inflammatory effects
Orthosiphols A and B, applied topically at 200 µg per ear, inhibited inflammation of mouse ears induced by 2 µg of 12-*O*-tetradecanoylphorbol-13-acetate by 42% and 50% respectively [23].

Effects on aortic contractile responses
Various substances isolated from the leaves of Java tea had a concentration-dependent suppressive effect on contractile responses in endothelium-denuded rat thoracic aorta strips. IC_{50} values were between 1.01×10^{-1} µmol/ml for acetovanillochromene and 8.08×10^{-3} µmol/ml for tetramethylscutellarein; nifedipine as a positive control gave a value of 1.79×10^{-5} µmol/ml [25,28]. Methylripariochromene A at doses of 1.1×10^{-5} M, 3.8×10^{-5} M and 1.1×10^{-4} M decreased the maximum contractions caused by Ca^{2+} at 30mM to 73.8%, 47.0% and 21.0% respectively [28,47].

Hypoglycaemic effects
A dried aqueous extract of Java tea (yield 3.3%) redissolved in saline was administered to rats by gavage at doses of 0.5 and 1.0 g/kg body weight. The control group received saline at 5 ml/kg body weight. In rats treated with an oral glucose load or streptozotocin, the extract produced a hypoglycaemic effect (no values given). The effect of the extract on streptozotocin-induced diabetes in rats was comparable to that of 10 mg/kg of glibenclamide [48].

Effects on blood pressure and heart rate
Methylripariochromene A at doses of 50 and 100 mg/kg body weight was administered subcutaneously to conscious, stroke-prone, spontaneously hypertensive male rats. A decrease of 15-30 mm Hg in mean systolic blood pressure was observed from 3.5 to 24 hours with the higher dose ($p<0.05$ and $p<0.01$ respectively), whereas no changes were observed in the control group. The lower dose caused a significant decrease ($p<0.05$) only at 8 hours. The substance also caused significant reductions ($p<0.01$) of 75 and 45 beats/min at 6.0 and 8.5 hours respectively after administration of the higher dose, initial values in the treatment groups being 334 (at 50 mg/kg) and 340 (at 100 mg/kg) beats/min, and in the control group 345 beats/min. A slight decrease in heart rate was observed 6 hours after administration of the lower dose; the heart rate returned to baseline after 24 hours [28,47].

Ex vivo experiments

Suppression of contractile force
After cumulative applications at 3.8×10^{-5} M and 1.2×10^{-4} M to spontaneously beating isolated guinea pig atria, methylripariochromene A significantly suppressed the contractile force by 18.8% ($p<0.05$) and 54.7% ($p<0.01$) respectively without significantly reducing the beating rate [28,47].

Pharmacological studies in humans

Diuretic activity
No influence on 12- or 24-hour urine output or sodium excretion was observed in 40 healthy volunteers after administration of 600 ml (3 × 200 ml at 4-hour intervals) of a decoction equivalent to 10 g of dried leaf in a placebo-controlled, double-blind crossover study [49].

A study carried out on 6 healthy male volunteers, who drank 4 × 250 ml of a decoction of Java tea at 6-hourly intervals during one day, for comparison with the same intake of water on a separate control day, acidity of the urine increased 6 hours after ingestion. There were no changes in urine volume or electrolytes [50].

From much earlier observations, however, increased diuresis was reported after oral administration of aqueous extracts of Java tea (400 ml/day of a 3.75% extract, 400 ml/day of a 15% extract, 500 ml/day of a 3.3% extract) to healthy volunteers [51-53].

Clinical studies

Diuretic activity
In an open study involving 14 patients, who received a 12% infusion of the leaves (500 ml/day for a period of 10 days), increased diuresis and elimination of chlorides and urea was reported [41].

A study in 67 patients suffering from uratic diathesis did not reveal any influence of Java tea on diuresis, glomerular filtration, osmotic concentration, urinary pH, plasma content or excretion of calcium, inorganic phosphorus and uric acid during 3 months of treatment [54].

Choleretic activity
Increased choleresis and cholekinesis, together with an antibacterial effect in cholecystitis and cholangitis, has been reported in patients after oral administration of a Java tea extract [55]. However, *in vivo* studies in rats with the isolated flavones sinensetin and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone administered intravenously at 10 mg/kg body weight did not confirm these findings [44].

Pharmacokinetic properties
No data available.

Preclinical safety data

In vitro experiments
Isolated diterpenes and flavonoids showed weak cytotoxicity towards murine colon carcinoma 26-L5 cells [29].

In a somatic segregation assay using the diploid strain *Aspergillus nidulans* D-30, no genotoxic effects (mitotic crossover, chromosome malsegregation, clastogenic damage) were detected after plate