Flos Trifolii

Definition
Flos Trifolii consists of the dried inflorescences of *Trifolium pratense* L. (Fabaceae) (*1*).

Synonyms
No information was found.

Selected vernacular names
Aasristik, aka kurooba, aka tsumekusa, akerklee, basim ahmar, beebread, broad-leaved clover, cow clover, creeping clover, hong san ye cao, hong hua san ye cao, hong che zhou cao, klever krasnyi, klever lugovoi, meadow clover, peavine clover, puna-apila, purple clover, red clover, red-klover, redo kurooba, ribah, rode klaver, rodklover, rödklöver, Rot-od-kopklee, Rothe Kleeblumen, Rother klee, Rother Wiesen-Klee, Rotklee, rod-klee, trébol, trébol common, trébol rojo, trébol violeta, tréfle common, tréfle des prés, tréfle rouge, tréfle violet, trefoil, trevor, trevo-dos-prados, trevo-violeto, trifoglio pratense, trifoglio violetto, wild red clover, Wiesen-Klee, wiesenklee (*2*–*8*).

Geographical distribution
Native to Europe. Found worldwide (*7*).

Description
A low-growing, common, perennial herb with ascending slender hairy stems bearing trifoliate leaves with broad, bristle-pointed stipules, the leaflets varying from ovate to obovate in outline, frequently notched at the apex, and showing a pale spot on their upper surface. The small butterfly-shaped flowers are borne in ovoid heads with long or short peduncles; their colour varies from magenta to whitish (*7*).

Plant material of interest: dried inflorescences

General appearance

Inflorescences are ovoid with a rounded summit, mostly from 12–34 mm in length and width, usually on a very short stalk, shrivelled, purplish, and more or less brown from drying, consisting of many papilionaceous flowers, crowded together and clothed at the base with broad, pointed, pale green ciliate stipules with darker veins. The flowers, which may or may not be accompanied by diminutive trifoliate leaves, are up to 15 mm in length and have the following: five green, hairy, subulate calyx teeth, one longer than the other four; petals united into a more or less campanulate tube, somewhat recurved, and colourless with pinkish purple veins; diadelphous stamens; slender style (*1*).

Organoleptic properties

Odour: faintly aromatic, somewhat tea-like; taste: sweetish, then slightly bitter (*1*).

Microscopic characteristics

Epidermis of calyx composed of polygonal cells with faintly striated cuticle and occasional anomocytic stomata on the outer epidermis only; abundant, uniseriate, covering trichomes with two small, thin-walled basal cells and a thick-walled tapering end cell, up to 1 mm in length with a warty cuticle. Glandular trichomes are also present, particularly on the lower epidermis, each with a one- or two-celled stalk and a large, cylindrical head composed of several cells arranged in two rows. Epidermal cells of the corolla, papillose at the tip, are elongated with slightly wavy walls and a strongly striated cuticle; vascular strands of corolla and calyx are surrounded by a crystal sheath containing prismatic crystals of calcium oxalate. The following are also present: fibrous layer of anthers; subspherical pollen grains, 20–48 mm in diameter with smooth exine, three distinct pores, and three furrows; upper epidermal cells of leaflets with sinuous and slightly beaded anticlinal walls; lower epidermis with sinuous to wavy walls; anomocytic stomata on both surfaces, but more frequent on the lower surface; abundant covering trichomes on both surfaces and on the margins; and fibrovascular strands surrounded by a crystal sheath containing prismatic crystals of calcium oxalate (*1*).

Powdered plant material

A pinkish-grey powder with a faint, fragrant odour and a slightly bitter taste. Fragments of corolla with slightly wavy walls and a striated cuticle; fragments of calyx with rectangular cells and a faintly striated cuticle; abundant uniseriate, warty-walled covering trichomes with part of the

end cell frequently broken off; glandular trichomes less frequent; subspherical pollen grains, scattered or associated with fragments of fibrous layer of anthers; abundant strands of vascular tissue with associated crystal sheath containing prismatic crystals of calcium oxalate; occasional portions of green leaf with wavy walled epidermis and anomocytic stomata (*3*).

General identity tests

Macroscopic and microscopic examination, as well as thin-layer and high-performance liquid chromatography (*1*, *3*).

Purity tests

Microbiological

Tests for specific microorganisms and microbial contamination limits are as described in the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*9*).

The United States pharmacopeia requires the absence of *Salmonella* species and *Escherichia coli*, with total aerobic microbial count not exceeding 10^6 colony-forming units (cfu) per g, the total combined moulds and yeast count should not exceed 10^4 cfu per g, and the enterobacterial count should not be more than 1000 cfu per g (*1*).

Foreign organic matter

Not more than 2% (*1*).

Total ash

Not more than 10% (*1*).

Acid-insoluble ash

Not more than 2% (*1*).

Water-soluble extractive

Not less than 15% (*1*).

Loss on drying

Not more than 12% (*1*).

Pesticide residues

The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (*10*). For other pesticides, see the *European pharmacopoeia* (*10*) and the WHO guidelines for assessing quality of herbal medi-

cines with reference to contaminants and residues (*9*) and pesticide residues (*11*).

Heavy metals

For maximum limits and analysis of heavy metals, consult the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*9*). The *United States pharmacopeia* stipulates total heavy metals not more than 10 µg/g (*1*).

Radioactive residues

Where applicable, consult the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*9*).

Other purity tests

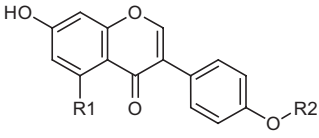
Chemical tests to be established in accordance with national requirements.

Chemical assays

Not less than 0.5% isoflavones, calculated on the dried basis as the sum of daidzein, genistein, formononetin and biochanin A (*1*).

Major chemical constituents

Rich in isoflavonoids. The major active estrogenic isoflavonoids are biochanin A, daidzein, formononetin and genistein (*5*, *13*, *14*, *17*). The structures of these isoflavonoids are presented below.



Daidzein	R1 = R2 = H
Formononetin	R1 = H R2 = CH_3
Genistein	R1 = OH R2 = H
Biochanin A	R1 = OH R2 = CH_3

Medicinal uses

Uses supported by clinical data

None.

Uses described in pharmacopoeias and well established documents

Although numerous clinical trials have assessed the safety and efficacy of red clover extracts for the treatment of menopausal symptoms, hyperlipidaemia, osteoporosis and prostate cancer (*15–19*), the data are as yet insufficient to support any of these indications. Further data from well-controlled clinical trials with sufficient numbers of subjects are needed before any therapeutic indications can be made.

Uses described in traditional medicine

Topical treatment of dermatological disorders such as psoriasis and eczema, as well as orally for the treatment of asthma and cough (*5*).

Pharmacology

Experimental pharmacology

Much of the experimental pharmacology for Flos Trifolii is based on information from pure compounds isolated from the crude drug, or from extracts of the aerial parts of the plant. Therefore, how these data apply to the crude drug needs to be further investigated.

Anti-inflammatory activity

Genistein has been found to provide protection from oxidative damage induced by ultraviolet (UV) radiation both in vitro and following dietary administration. One in vivo study assessed the potential of a number of isoflavones isolated from the crude drug, as well as some metabolically related compounds, to offer protection against UV irradiation. The results were assessed in hairless mice after topical application of the extract or isoflavones in combination with UV exposure. Daidzein, biochanin A and formononetin were not active, while 20 µM lotions of genistein and the metabolites equol, isoequol and dehydroequol reduced oedema and inflammation, as well as suppressing hypersensitivity induced by moderate doses of artificial UV radiation. The protective effect of equol was concentration-dependent and 5 µM equol markedly reduced UV-induced inflammation (*20*).

Inhibition of cell proliferation

Isoflavones inhibit the growth of some types of tumour cells, including prostate adenocarcinoma. In the prostate cancer cell line, LNCaP, and xenografts, the mechanism of the antiproliferative effects of biochanin A was determined (*18*). LNCaP cells were treated with varying concentrations of biochanin A to evaluate viability, DNA synthesis and DNA fragmentation by terminal deoxynucleotidyltransferase dUTP nick end labelling (TUNEL) analysis. Regulation of gene expression was determined using Western immunoblotting and cDNA microarrays. Antiproliferative effects were evaluated by using athymic mice with LNCaP flank tumours. Biochanin A induced a dose-dependent inhibition of LNCaP cell proliferation and tritiated thymidine incorporation that correlated with increased DNA fragmentation, indicative of apoptosis. Western blot analyses of cell cycle regulatory proteins revealed that biochanin A significantly decreased expression of cyclin B and p21, whereas flow cytometry showed that cells were accumulating in the G(0)/G(1) phase. cDNA microarray

analyses identified 29 downregulated genes with six reduced below assay detection limits. Eleven genes were upregulated, including nine that were undetectable in controls. In mice with LNCaP xenografts, biochanin A significantly reduced tumour size and incidence (*18*). In a similar study, intragastric administration of an extract of the flowers to aromatase knockout mice reduced the enlargement of a non-malignant prostate (*21*). A study examined the effect of dietary isoflavones on prostate growth in intact male mice treated with an unidentified extract of red clover. The results demonstrated that prostate, but not testis, size was significantly reduced over 28 days of being fed a diet supplemented with red clover isoflavone. Histological examination revealed an increase in apoptotic cells, rather than a reduction in proliferative activity in the epithelium. These findings support the hypothesis that red clover isoflavones in the diet can induce apoptosis and lead to a reduction in prostate size (*22*).

Estrogenic effects

The estrogenic effect of a standardized ethanol extract, with an average isoflavone content of approximately 9% (dry weight), was assessed in vitro in a yeast two-plasmid system expressing estrogen receptor alpha and estrogen receptor beta. The extract had an estrogenic activity that corresponded to a transactivational capacity of approximately 18 µg of 17-β-estradiol/g extract for estrogen receptor alpha and approximately 78 µg 17-β-estradiol/g extract for estrogen receptor beta. The difference is due to the higher binding affinity of the isoflavone constituents to estrogen receptor beta than that observed for estrogen receptor alpha (*23*).

A methanol extract (15% total isoflavones) of red clover (*Trifolium pratense* L.) showed significant competitive binding to estrogen receptor alpha and estrogen receptor beta in vitro ($p < 0.01$). In Ishikawa (endometrial cancer) cells, the extract also exhibited estrogenic activity as indicated by induction of alkaline phosphatase activity and upregulation of progesterone receptor mRNA. In S30 breast cancer cells, presenelin-2, another estrogen-inducible gene, was upregulated in the presence of red clover. Bioassay-guided isolation utilizing estrogen receptor competitive binding as a monitor, and screening using ultrafiltration liquid chromatography–mass spectrometry revealed that genistein was the most active component of red clover, and the most effective of four red clover isoflavones tested in the above in vitro assays (*24*).

An extract of the crude drug (15% total isoflavones) bound to the alpha and beta estrogen receptors with a median inhibitory concentration of 18 and 2 µg/ml, respectively. The extract activated the estrogen response element in Ishikawa cells and induced luciferase expression in MCF-7 cells (*25*).

An in vivo study assessed the estrogenic effects of the crude drug in ovariectomized rats (*25*). A red clover extract, standardized to contain 15% isoflavones was administered by gavage, at a dose of 250, 500 or 750 mg/kg body weight (bw) per day, to virgin, ovariectomized 50-day-old rats, for 21 days in the presence and absence of 17-β-estradiol (50 µg/kg bw per day). The estrogenic effects assessed included an increase in uterine weight, vaginal cell cornification and branching of mammary gland ducts. The extract of the flowers produced a dose-dependent increase in uterine weight and differentiated vaginal cells at the two higher doses, but it did not stimulate cell proliferation in the mammary glands. Neither antiestrogenic nor additive estrogenic properties were observed in any of the tissues studied. These data suggest that red clover extract is weakly estrogenic in ovariectomized rats (*26*).

Thyroid effects

The effects of isoflavones on the secretion of thyroid hormones as well as on the immunoreactivity to estrogen receptor alpha in the thyroid glands of ovariectomized ewes were studied. Eight ewes were fed 3.5 kg of 100% red clover silage daily for 14 days. Blood samples were collected before and on day 14 of exposure to phytoestrogens. After 5 months, four of the ewes were re-exposed to red clover silage as described above and the other four served as controls. Ewes exposed to red clover silage had significantly higher plasma concentrations of total T(3) and free T(3) than ewes fed hay. The cross-sectional area of thyroid follicles tended to be larger in ewes fed red clover silage than in the control animals. Estrogen receptor alpha immunoreactivity was stronger in thyroid glands from ewes exposed to phytoestrogens than in ewes fed hay. Daily ingestion of 81–95 mg phytoestrogens per kg bw for 14 days stimulated secretion of thyroid hormones and tended to increase follicle size and estrogen receptor alpha immunoreactivity of thyroid glands (*27*).

Clinical pharmacology

A randomized, double-blind, placebo-controlled, cross-over trial involving 51 perimenopausal and postmenopausal women assessed the effects of an extract of the crude drug for the treatment of hot flushes (*28*). The subjects had been amenorrhoeic for at least 6 months and had at least three hot flushes per day. The women received one tablet of either placebo or the extract (containing 40 mg total isoflavones, including genistein, 4.0 mg; daidzein, 3.5 mg; biochanin, 24.5 mg; and formononetin, 8 mg). Phase one of the trial lasted for 3 months, and was followed by a 1-month washout period. The subjects were then crossed over to the other arm for a further 14 weeks. All subjects were required to maintain a diary of

symptoms based on the Greene Menopause Score list. Of the initial 51 subjects entering the trial, 43 completed the study. At 12 weeks the frequency of hot flushes had decreased in both the group receiving placebo and in the group receiving the treatment, by 18% and 20%, respectively. However, there were no statistically significant differences between groups in frequency of hot flushes or Greene Scores at any time point. No significant changes in body weight, steroid hormone binding globulin levels, blood counts, serum electrolytes, urea, creatinine or liver function were observed (*28*).

A second randomized, double-blind, placebo-controlled trial involved 37 perimenopausal and postmenopausal women and also assessed the effects of an extract of the crude drug for the treatment of hot flushes (*15*). All subjects had been amenorrhoeic for at least 6 months and had at least three hot flushes per day. The women were randomly assigned to receive either one tablet of placebo or one of two doses of an extract (containing 40 mg or 160 mg of total isoflavones; the 40-mg tablet containing genistein, 4.0 mg; daidzein, 3.5 mg; biochanin, 24.5 mg; and formononetin, 8 mg) for 12 weeks. The outcomes measured were similar to those of the previous study. During the 12-week treatment period, the frequency of hot flushes was reduced by 35% in the placebo group; by 29% in the group treated with 40 mg; and 34% in the group treated with 160 mg of the extract. No significant changes in body weight, levels of steroid hormone binding globulin, blood counts, serum electrolytes, urea, creatinine or liver function were observed (*15*).

A third randomized, placebo-controlled trial involving 30 menopausal women who had had amenorrhoea for more than 12 months, and who were also experiencing more than five hot flushes per day, assessed the effect of the same extract as described in the previous two studies for the treatment of menopausal symptoms. All subjects participated in a single-blind phase in which they received placebo tablets for 4 weeks, and then they were randomly assigned to receive either placebo or 80 mg isoflavones for a further 12 weeks. Efficacy was measured by the decrease in number of hot flushes per day and changes in the Greene Menopause Scale score. During the first 4 weeks of treatment with the placebo, the frequency of hot flushes decreased by 16%. During the subsequent double-blind phase, a further, statistically significant decrease of 44% was seen in the group treated with 80 mg of isoflavones ($p < 0.01$), whereas no further reduction occurred in women in the group treated with the placebo. The Greene Menopause Scale score decreased by 13% in the group treated with the isoflavones and remained unchanged in the women treated with the placebo (*29*).

A fourth study compared the efficacy and safety of two products derived from red clover with that of a placebo in symptomatic menopausal women. This randomized, double-blind, placebo-controlled trial involved menopausal women, aged 45–60 years, who were experiencing at least 35 hot flushes per week. The study included women who had recently become postmenopausal (mean (standard deviation), 3.3 (4.5) years since menopause) experiencing on average 8.1 hot flushes per day. Women were excluded from the study if they were vegetarians, consumed soy products more than once per week, or took medications affecting isoflavone absorption. After a 2-week placebo run-in, 252 participants were randomly assigned to receive either Promensil (82 mg of total isoflavones per day), Rimostil (57 mg of total isoflavones per day) or a placebo identical in appearance to the clover products, and followed up for 12 weeks. The primary outcome measured was the change in frequency of hot flushes as recorded by participants in their daily diaries. Secondary outcome measures included changes in quality of life and adverse events. Of 252 participants, 246 (98%) completed the 12-week protocol. The reductions in mean daily count of hot flushes at 12 weeks were similar in all three groups: Promensil, 5.1; Rimostil, 5.4; and placebo, 5.0. In comparison with the group treated with the placebo, participants who received the Promensil (41%; 95% confidence interval (CI), 29%–51%; $p = 0.03$), but not those treated with Rimostil (34%; 95% CI, 22%–46%; $p = 0.74$) found that the treatment reduced hot flushes more rapidly. Improvements in quality of life and reports of adverse events were comparable in all three groups (*19*).

Data from two clinical trials, published only as abstracts, are also available (*30*). A randomized, double-blind, placebo-controlled pilot study conducted in Peru assessed the efficacy of a red clover product in 30 postmenopausal women. The women were treated with 40 mg of the extract or a placebo for 4 months (*31*). A 75% reduction in hot flushes occurred in the treatment group; however, no data for the placebo group were reported. The second study was an uncontrolled trial (no placebo group) in 23 women with ammenorrhoea for 12 months (*32*). In patients treated with 40.0 mg of the extract, there was a 56% reduction in the frequency of hot flushes over a 2-month period. The severity of hot flushes decreased by 43% and the severity of night sweats decreased by 52%. No changes in endometrial thickness and no adverse effects were observed. Complete blood counts were all within normal limits (*32*).

The effects of a red clover-derived isoflavone extract on the Ki-67 proliferative marker of endometrial biopsies were assessed in a double-blind, randomized, controlled study involving 30 perimenopausal women. The

purpose of the study was to detect a decrease in the Ki-67 proliferative index during the late follicular phase after a 3-month course of 50.0 mg/day red clover isoflavones. The biopsies were timed as close as possible to days 7–11 of the menstrual cycle, and simultaneous measurements of trans-vaginal endometrial thickness, uterine artery Doppler, hormone profiles, lipids and bone markers were made. Of 30 women, two did not return for a second biopsy, and a third had an unsuccessful second biopsy. Four subjects were excluded from the intention-to-treat analysis because they did not have menstrual bleeding within the time frame of the study (3 subjects) or were tested on day 13 instead of between days 7 and 11 of the cycle (1 subject). There was no change in the Ki-67 proliferation index after treatment in either group. Eight women in the group given the placebo and eight in the treatment group had proliferative endometrial biopsies that were synchronized with estradiol levels at baseline and post-treatment, and analysis of these subjects revealed no detectable change in the relationship between estradiol levels and Ki-67 with treatment in either group. There was no change in fasting lipids, bone markers, uterine Doppler resistance or pulsatility index (*33*).

Effects on bone and cardiovascular system

Six trials have assessed the effects of isoflavones on total cholesterol and lipid levels (*27*, *34–38*). A randomized, single-blind, cross-over study involving 21 premenopausal women with regular menstrual cycles assessed the effect of an extract of the crude drug on plasma lipids and oxidization of low-density lipoprotein cholesterol (*34*). Subjects were treated with a placebo or 86 mg of isoflavones per day for two menstrual cycles, after which they were crossed over to the other group. Fourteen women completed the study and no differences in total cholesterol, triglycerides or oxidized low-density lipoprotein were observed.

A randomized, double-blind, placebo-controlled study involving 66 postmenopausal women with hypercholesterolaemia assessed the effects of an ascending dose of the extract, 40 or 80 mg of isoflavones, on lipid profiles (*35*). Study participants were asked to follow a low isoflavone diet for 3 months during the study. Treatment did not affect total cholesterol, low-density lipoprotein, high-density lipoprotein cholesterol or plasma triglycerides at any dose.

A double-blind placebo-controlled trial compared the effects of two doses of isoflavones, 40 and 80 mg, with the effects of a placebo on arterial compliance and plasma lipids in menopausal women. After a 3–4-week run-in period and a 5-week placebo phase, 26 women were randomly allocated to receive either 40 mg of the extract or the placebo for an additional 5 weeks, and then the dose was increased to 80 mg. Sixteen

women completed the trial. The results demonstrated a significant improvement in arterial compliance at both the 40- and 80-mg doses as compared with placebo ($p < 0.05$). No significant differences were seen between the two doses (*30*, *36*).

A double-blind study to evaluate the effects of varying doses of isoflavones on lipid and bone metabolism in postmenopausal women was performed. An extract of red clover, containing genistein, daidzein, formononetin and biochanin was administered to 46 postmenopausal women after a single-blind placebo phase; this was followed by a single-blind wash-out phase. Patients were randomly assigned to receive 28.5 mg, 57 mg or 85.5 mg of isoflavones daily for 6 months. At 6 months, the serum high-density lipoprotein cholesterol was found to have risen significantly by 15.7–28.6% with the different doses ($p = 0.007$, $p = 0.002$ and $p = 0.027$ at doses of 28.5 mg, 57 mg and 85.5 mg, respectively), although the magnitude of the response was independent of the dose used. The serum apo-lipoprotein B fell significantly by 11.5–17.0% with the different doses ($p = 0.005$, $p = 0.043$, $p = 0.007$, respectively) and the magnitude of the response was independent of the dose used. The bone mineral density of the proximal radius and ulna rose significantly by 4.1% over 6 months in the women who received 57 mg/day ($p = 0.002$) and by 3.0% in the women who received 85.5 mg/day ($p = 0.023$) of isoflavones. The response to treatment with 28.5 mg/day of isoflavones was not significant. These results show that the administration of an isoflavone combination extracted from red clover was associated with a significant increase in high-density lipoprotein cholesterol, a significant fall in apolipoprotein B, and a small but significant increase in the predominantly cortical bone of the proximal radius and ulna after 6 months of treatment (*37*).

A double-blind, placebo-controlled study, involving 107 premenopausal, perimenopausal and postmenopausal women assessed the effects of an extract of the crude drug (corresponding to 40 mg of isoflavones per day) for 1 year on bone mineral density and content (*39*). The outcomes measured were changes in the lumbar spine and total hip bone mineral content and density. After 1 year, the bone mineral content and density of the lumbar spine (measured by dual energy X-ray absorptiometry (DEXA) scan) had decreased significantly ($p < 0.01$) in both groups. However, the decrease in the bone mineral content and density of the spine of premenopausal and perimenopausal women treated with the extract was significantly lower than that in women who received the placebo ($p < 0.02$ and $p < 0.01$, respectively). No differences were observed in the postmenopausal women. No differences in bone mineral density of the

hip or significant changes in markers of bone turnover were seen in any of the groups (*39*).

A 12-week randomized, double-blind, placebo-controlled trial was conducted involving 252 menopausal women aged 45–60 years. The women, who were experiencing > 35 hot flushes per week, were randomly assigned to receive either Promensil (82 mg total isoflavones), Rimostil (57.2 mg total isoflavones) or a placebo. The primary outcome measures were mean absolute changes for high-density lipoprotein cholesterol, serum osteocalcin and urinary N-telopeptide. The secondary outcome measures were mean changes of total cholesterol, low-density lipoprotein cholesterol, the ratio of high-density lipoprotein cholesterol to low-density lipoprotein cholesterol, and triglycerides. Ninety-eight per cent of the participants completed the 12-week protocol. Women who took Rimostil or Promensil had greater mean increases in high-density lipoprotein cholesterol than those who took placebo; however, this change was small (< 2 mg/dl) and was not statistically significant. There was a significant decrease in triglyceride levels among women who took Rimostil (14.4 mg/dl) or Promensil (10.9 mg/dl) compared to those who took the placebo. The decrease was seen primarily among women with elevated baseline triglyceride levels. There were no differences in mean changes of total cholesterol, low-density lipoprotein cholesterol, or the ratio of high-density lipoprotein cholesterol to low-density lipoprotein cholesterol among treatment groups. There were no statistically significant differences between treatment groups for bone turnover markers. It was concluded that compared with placebo, extracts containing isoflavones decrease levels of triglycerides in symptomatic menopausal women; however, this effect was small (*38*).

Treatment of prostate cancer

A nonrandomized, non-blinded trial with historically matched controls from archival tissue assessed the effects of acute exposure to a dietary supplement of isoflavones in men with clinically significant prostate cancer before radical prostatectomy. Thirty-eight patients were recruited to the study upon diagnosis of prostate cancer. Before surgery, 20 of the men consumed 160 mg/day of red clover-derived dietary isoflavones, containing a mixture of genistein, daidzein, formononetin and biochanin A. Serum prostate-specific antigen, testosterone and biochemical factors were measured, and clinical and pathological parameters were recorded. The incidence of apoptosis in prostate tumour cells from radical prostatectomy specimens was compared between 18 treated and 18 untreated control tissues. There were no significant differences between pretreatment and post-treatment serum prostate-specific antigen, Gleason score, serum

testosterone or biochemical factors in the treated patients ($p > 0.05$). Apoptosis in radical prostatectomy specimens from treated patients was significantly higher than in control subjects ($p = 0.0018$), specifically in regions of low to moderate-grade cancer (Gleason grade 1–3). No adverse events related to the treatment were reported (*18*).

Pharmacokinetics and pharmacodynamics

The pharmacokinetics of red clover isoflavones were investigated after long-term administration as a once-daily dietary supplement. Fourteen subjects who had been consuming a low-isoflavone containing diet for 2 weeks were given an oral dose of two isoflavone tablets (approximately 80 mg of total isoflavones) daily for 2 weeks. Before the study day the participants fasted overnight and were then administered their last dose the next morning. Plasma samples were collected for a 48-hour period after the last dose. Plasma isoflavones were assayed by high-performance liquid chromatography. The results demonstrate that trough plasma levels were significantly higher for daidzein and genistein after long-term dosing than levels recorded prior to the commencement of the study and plasma levels of isoflavones after long-term dosing were in the range previously reported in populations that consume an isoflavone-rich diet. The plasma half-lives observed after long-term administration were consistent with once-daily administration. Isoflavones have pharmacokinetic characteristics that suggest that once-daily administration is adequate when they are administered long-term (*40*).

The absorption of isoflavones varies substantially between individuals. A single-blind, randomized, placebo-controlled, cross-over trial involving 14 subjects investigated whether isoflavone absorption differs depending on whether the isoflavones originated from soy beans or from red clover. Soy bean isoflavone glycosides and red clover isoflavone aglycones were incorporated into a breakfast cereal and eaten daily for 2 weeks each, separated by a 2-week control or washout period. The excretion of isoflavones in urine was measured over a 24-hour period; approximately 25% of each isoflavone was recovered in urine, suggesting that similar amounts were absorbed irrespective of their glycoside/aglycone nature or the differing compositions of their sources (daidzein and genistein in soy beans and formononetin and biochanin in red clover). Although interindividual variability was high, there was less intraindividual variability; the amounts excreted when subjects consumed the two sources of isoflavone were correlated ($r = 0.69$; $p = 0.007$) (*41*).

Adverse reactions

Ingestion of large amounts of clover in animal feed has been associated with a number of adverse effects in sheep in Australia. A publication on "clover disease" described symptoms of infertility, abnormal lactation, dystocia and prolapsed uterus, all of which were hypothetically attributed to the estrogenic effects of isoflavones (*42*). None of the controlled, clinical trials has reported adverse effects at doses up to 160 mg of isoflavones per day. *Trifolium pratense* does not contain coumarins, and therefore the concerns about blood coagulation are unfounded (*43*, *44*).

Contraindications

Flos Trifolii is contraindicated in cases of hypersensitivity or allergy to the crude drug. It is also contraindicated during pregnancy, breastfeeding and for children under the age of 12 years, and in cases of hormone-associated diseases, due to the potential hormonal effects.

Warnings

Due to the potential estrogenic effects of the crude drug, patients with hormone-related disorders, estrogen-dependent cancers or a familial history of estrogen-dependent cancers should contact a health care provider before use.

Precautions

Carcinogenesis, mutagenesis, impairment of fertility

No information was found.

Drug interactions

There are conflicting data concerning an interaction of the crude drug with tamoxifen and other antiestrogenic drugs. Some studies suggest that specific isoflavones may enhance the ability of tamoxifen to inhibit the growth of estrogen-receptor-positive breast cancer cells (*45–47*). In rodent models, genistein has been shown to inhibit the efficacy of tamoxifen on the growth of estrogen-receptor-positive breast cancer cells implanted in ovariectomized mice, while other research shows that specific isoflavones may be additive and work in a synergistic manner to prevent the development of chemically induced tumours and the growth of existing tumours (*48–50*). Therefore, the use of the crude drug or its preparations is not recommended in those being treated with tamoxifen and other antiestrogenic drugs until further research has been done.

Pregnancy: non-teratogenic effects
See Contraindications.

Nursing mothers
See Contraindications.

Paediatric use
See Contraindications.

Dosage forms
Crude drug and tablets.

Posology
(Unless otherwise indicated)
Oral dose: extracts of crude drug: 240–480 mg corresponding to 40–80 mg/day of isoflavones (*15*, *17*, *28*, *29*).

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