

## Red Clover (*Trifolium pratense*)

Natural Standard Monograph ([www.naturalstandard.com](http://www.naturalstandard.com)) Copyright © 2009.



### Synonyms/Common Names/Related Substances:

- Ackerklee (German), beebread, cow clover, genistein, isoflavone, meadow clover, phytoestrogen, Promensil®, purple clover, Rotklee (German), Rimostil®, trefoil, trefle des pres (French), trifolium pratense, Trinovin®, wild clover.

### CLINICAL BOTTOM LINE/EFFECTIVENESS

#### Brief Background:

- Red clover is a legume, which like soy contains "phytoestrogens" (plant-based compounds structurally similar to estradiol, capable of binding to estrogen receptors as an agonist or antagonist). Red clover was traditionally used to treat asthma, pertussis, cancer, and gout. In modern times, isoflavone extracts of red clover are most often used to treat menopausal symptoms, as an alternative hormone replacement therapy, for hyperlipidemia, or to prevent osteoporosis.
- At this time, there are no high-quality human trials supporting the efficacy of red clover for any indication (1). Soy protein, another source of isoflavones, has been reported to significantly reduce serum lipid levels, but this benefit has not been demonstrated for red clover, and may be due to the presence of other constituents in soy (saponins, pectins, essential fatty acids).

#### Scientific Evidence for Common/Studied Uses:

Indication	Evidence Grade	 GRADING SYSTEM LINK
Menopausal signs and symptoms	C	
Hormone replacement therapy (HRT)	C	
Osteoporosis	C	
Hypercholesterolemia	C	
Benign prostatic hyper trophy (BPH)	C	

Prostate cancer	C	

#### Historical or Theoretical Indications that Lack Sufficient Evidence:

- Acne, AIDS, antibacterial, antioxidant (2), antispasmodic, appetite suppressant, arthritis, asthma, "blood purification," bronchitis, burns, cancer prevention (antitumor), canker sores, cough, chronic skin diseases (topical), diuretic, eczema (topical), gout, hypertension (3), indigestion, mastalgia (4), premenstrual syndrome, psoriasis (topical), sexually transmitted diseases (STDs), skin ulcers/sores (topical), sore eyes, tuberculosis, venereal diseases, whooping cough (pertussis).

#### Expert Opinion and Folkloric Precedent:

- Chinese and Russian folk healers have used red clover to treat respiratory problems such as asthma and bronchitis. Native American healers recommended red clover for pertussis and cancer.
- Some experts believe that topical red clover accelerates wound healing and alleviates psoriasis. Recently, it has been speculated that red clover may have beneficial effects on bone metabolism, serum lipid levels, and arterial compliance, due to its phytoestrogen properties. Red clover is often recommended for these indications, although there is a paucity of scientific evidence.

#### Brief Safety Summary:

- Likely Safe:** When used in recommended doses as a supplement for the relief of menopausal symptoms and as an adjunctive preventative therapy for osteoporosis (5;6;7). Small trials have not noted significant adverse effects after one year of red clover isoflavone therapy (8;9).
- Possibly Safe:** Caution should be taken with patients on hormone replacement therapy (HRT) or oral contraceptives (OCPs), as red clover binds to intracellular estrogen receptors and may enhance estrogenic effects (10).
- Possibly Unsafe:** Red clover may alter platelet aggregation/contains coumarin, and therefore may theoretically be unsafe in persons with bleeding disorders/coagulopathies or taking anticoagulants. Caution should be exercised in patients with/at risk for breast cancer/estrogen receptor-positive neoplasia, due to the variable estrogen receptor binding properties of red clover (may have estrogen agonist or antagonist properties with unclear long-term effects). In theory, estrogenic activity unopposed by progesterone may lead to endometrial hyperplasia, and ultimately may increase risk of uterine (endometrial) cancer, although short-term studies (<6 months) have found no visible changes on ultrasound (11;12). It remains unclear if phytoestrogens such as red clover affect these risks, and further research is warranted in these areas.

## General:

- Recommended doses are based on those most commonly used in available trials, or on historical practice. However, with natural products it is often not clear what the optimal doses are to balance efficacy and safety. Preparation of products may vary from manufacturer to manufacturer, and from batch to batch within one manufacturer. Because it is often not clear what are the active components of a product, standardization may not be possible, and the clinical effects of different brands may not be comparable.

## Standardization:

- Isoflavones are isolated from red clover via alcohol extraction. The brand of red clover isoflavone extract used in most trials, and which is most commonly available commercially, is Promensil® (Novogen). Each tablet is standardized to contain 40mg total isoflavones: 4mg genistein; 3.5mg daidzein; 24.5mg biochanin A; and 8.0mg formononetin (present as hydrolyzed aglycones).
- Methods used to identify constituents of red clover include high-performance liquid chromatography (HPLC) (13), and more recently polymerase chain reaction (PCR) techniques (14).

## Adult Dosing (18 years and older):

### Oral:

- **Note:** Recommended doses are based on those most commonly used in available trials.
- **Hormone Replacement:** 40-80mg of red clover isoflavones/day (Promensil®) (7;15;11;16;12).
- **Hypercholesterolemia:** 28.5mg, 57mg, or 85.5mg of red clover isoflavones/day (Rimostil®) (5), or: 80mg of red clover isoflavones/day (Promensil®) (17).
- **Osteoporosis:** 40mg of red clover isoflavones/day (Promensil®) (5).
- **Menopausal Symptoms:** 40mg, 80mg, or 160mg of red clover isoflavones/day (Promensil®) (11;6).
- **Benign Prostatic Hypertrophy:** 40mg of red clover isoflavones/day (Trinovin®) (18).

## Pediatric Dosing (younger than 18 years):

- Insufficient available evidence to recommend.

## Toxicology:

- Insufficient human data available. In grazing animals, red clover has been noted anecdotally to be

associated with cachexia, bloating, and abortion.

---

## PRECAUTIONS/CONTRAINDICATIONS

### Pregnancy & Lactation:

- Red Clover often is not recommended during pregnancy and lactation due to its estrogenic activity (10).
- One small rat study found that maternal exposure to subcutaneous genistein (an isoflavone) increases the incidence of mammary tumors in offspring, mimicking the effects of *in utero* estrogenic exposures (22).
- Red clover has been implicated as a cause of infertility and abortion in grazing livestock.

---

## INTERACTIONS

- Hormone Replacement Therapy (HRT), Oral Contraceptives (OCPs), Tamoxifen, Raloxifene:** Red clover isoflavones possess varying affinity for estradiol receptors (estradiol- $\alpha$  and estradiol- $\beta$ ), and are capable of acting as both agonists and antagonists (20). Preliminary evidence suggests a preferential binding to estrogen receptor  $\beta$ , which is found in the vasculature, brain, bone, and heart, as opposed to estrogen receptor  $\alpha$  (found in the ovaries, breast, uterus, and adrenal glands). Concomitant use of red clover may enhance or inhibit the estrogenic effects of hormonal therapies. Isoflavones may affect levels of gonadotropin releasing hormone (GrRH), follicle stimulating hormone (FSH), and leutinizing hormone (LH) via hormonal feedback mechanisms (10).

---

## MECHANISM OF ACTION

### Pharmacology:

- **Estrogenic properties:** Although red clover has been found to contain over 125 chemical compounds, the isoflavone component of the plant is thought to be responsible for the majority of its therapeutic applications. Isoflavones, like those found in red clover, are considered to be "phytoestrogens." Phytoestrogens are plant compounds that are structurally similar to estradiol and are capable of binding to estradiol receptors. Isoflavones have a varying affinity for estradiol receptors (estradiol- $\alpha$  and estradiol- $\beta$ ), and are capable of acting as both agonists and antagonists (20). There is some evidence that suggests a preferential binding to estrogen receptor  $\beta$ , which is found in the vasculature, brain, bone, and heart, as opposed to estrogen receptor  $\alpha$  (found in the ovaries, breast, uterus, and adrenal glands).

- As an estrogen agonist, red clover may lead to the inhibition of gonadotropin releasing hormone (GrH), follicle stimulating hormone (FSH), and leutinizing hormone secretion via negative feedback (10). However, the clinical significance of this activity in humans has not been determined. The constituent isoflavones biochanin A and genistein have been reported to have relatively high levels of estrogenic activity. Daidzein appears to be less active (23). However, biochanin A, formononetin, genistein and diadzein do not possess observable progestational or androgenic effects (23), and in fact have been proposed to possess anti-androgen properties (24). Red clover has demonstrated estrogenic activity via the induction of alkaline phosphatase activity and up-regulation of progesterone receptor mRNA (25), with physiological effects observed in animal research (26).
- Antiproliferative properties:** Biochanin A and genistein have been shown to inhibit cell proliferation *in vitro* (apoptosis is believed to be the primary mechanism of action, based on demonstrated DNA fragmentation) (27;28;29). Genistein inhibits cell proliferation via inhibition of tyrosine kinases and DNA topoisomerases I and II (20). It has been speculated that genistein's cytotoxicity may be the result of disruption of transcriptional processes (30). Coumestrol is a phytoestrogen found in high quantities in red clover. *In vitro*, coumestrol has been shown to exhibit both mutagenic and clastogenic (chromosomal breaking) properties in cultured human lymphoblastoid cells (31). Anti-androgenic properties have also been proposed as possibly beneficial in cases of prostatic hypertrophy or prostate cancer (24).
- Antioxidant properties:** Red clover's purported antioxidant properties have been attributed to the isoflavone genistein. Genistein inhibits the formation of hydrogen peroxide and superoxide anion, and scavenges hydrogen peroxide *in vitro*. Daidzein also appears to possess antioxidant properties, although to a lesser extent (28).

#### Pharmacodynamics/Kinetics:

- Red clover differs from the phytoestrogen soy in that the principal isoflavones in red clover are biochanin A and formononetin, while those in soy consist solely of genistein and diadzein. However, biochanin A and formononetin are metabolized extensively *in vivo* to genistein and diadzein respectively (19). Daidzein is further broken down to form the estrogenic metabolite equol and the less estrogenic O-desmethylangolensin (O-DMA) (32). Metabolism and absorption (which may be influenced by food) are highly variable (32).
- Genistein, daidzein, and equol have been detected in human urine, plasma, saliva, breast aspirate and prostatic fluid (20).
- The plasma half-life of daidzein and genistein is approximately 7.9 hours in adults; peak plasma concentrations occur 6-8 hours after oral administration of the pure compound (33).
- Although isoflavones are structurally related to estrogen, they have a variable affinity for the estradiol binding site, and may act as either agonists or antagonists (20).

- Historically, red clover has played a role in medicine, agriculture, and religion. Chinese and Russian folk healers used it to treat respiratory conditions such as asthma and bronchitis. In the Middle Ages, it was considered a charm against witchcraft. Red clover serves as a grazing food for many animals, and has been implicated as a cause of infertility in livestock. Some believe that topical red clover can accelerate wound healing and alleviate psoriasis.

## EVIDENCE TABLE

Condition	Study Design	Author, Year	N	Statistically Significant?	Quality of Study 0-2=poor 3-4=good 5=excellent	Magnitude of Benefit	ARR	NNT	Comments
Menopausal symptoms	Randomized, placebo controlled (RCT), crossover, single-blinded	Baber, 1999	51	No	2	None	NA	NA	No benefit of Promensil vs. placebo, but no power calculation done: sample may have been too small to detect benefit.
Menopausal symptoms	RCT	Knight, 1999	37	No	2	NA	NA	NA	No benefit of Promensil vs. placebo. Isoflavone ingestion in placebo group may have confounded results. No power calculation done.
Menopausal symptoms	RCT, double-blind	Jeri, 1999	30	Yes	2	Large	53%	2	Conference abstract only (poorly reported). Significant reduction in hot flash frequency vs. placebo.
Menopausal symptoms	RCT, double-blind	Van de Weijer, 2001, 2002	30	Yes	2	Mixed	NA	NA	Conference abstract only. 44% decrease in median # of hot flashes, but unclear statistical significance vs. placebo. No change in subjective symptom severity.
Menopausal symptoms	Case series	Nachtigall, 1999	23	Yes	NA	Large	NA	NA	Improvements in hot flashes and night sweats, but lack of controls limits usefulness of results.
Menopausal symptoms	Case series	Abernathy, 2001	33	Yes	NA	Mixed	NA	NA	44% reduction in hot flash frequency, but no change in subjective symptoms. Lack of controls limits

									usefulness of results.
Arterio-vascular resistance	RCT	Nestel, 1999	26	No	2	NA	NA	NA	Inappropriate randomization, data collection from 16 participants only.
Bone density and lipid metabolism	Case series (dose escalation study)	Clifton-Bligh, 2001	46	Yes	0	NA	NA	NA	Rimostil superior to placebo. No control group or dietary log to rule out increases in isoflavone enriched foods.
Bone mineral density	RCT, double-blind	Atkinson, 2000	107	Yes	2	Mixed	NA	NA	Improvement in pre and peri-menopausal women with Promensil, but not in post-menopausal. Conference abstract only with limited details.
Bone mineral density	Case series (dose escalation study)	Baber, 1999	50	Yes	2	Small	NA	NA	Improved bone density in some forearm areas with Promensil vs. placebo (3%). Conference abstract only, 8 dropouts.
Hyper-cholesterolemia	RCT, double-blind	Howes, 2000	93	No	3	NA	NA	NA	Small study with high dropout rate: 18 dropouts.
Hyper-cholesterolemia	RCT, double-blind	Blakesmith, 2003	25	No	2	None	NA	NA	86mg isoflavones/day. Small study in healthy premenopausal women. Increased urinary isoflavones without changes in LDL or HDL. May have been underpowered.
Hyper-cholesterolemia	RCT, single-blind, crossover	Samman, 2000	21	Yes	1	None	NA	NA	86mg isoflavones/day no better than placebo, but 33% dropout and no power calculation.
Benign prostatic hypertrophy	Case series	Gerber, 2000	29	Yes	NA	NA	NA	NA	Trinovin decreased BPH symptoms. Abstract only. Study funded by manufacturer.

## Menopausal Signs and Symptoms

- **Summary:** Isoflavones, such as those present in red clover, are believed by many experts to reduce signs and symptoms of menopause (such as hot flashes). Although the isoflavones present in red clover have been demonstrated to possess estrogenic properties in pre-clinical studies, there is currently insufficient evidence demonstrating efficacy or safety in humans. Trials have been methodologically weak and short in duration ( $\leq 12$  weeks of treatment), which may not be sufficient to assess efficacy for menopausal symptoms, which tend to wax and wane over longer periods of time. Nonetheless, red clover products remain popular.
- **Randomized trials:** Baber et al. conducted a randomized, placebo controlled, crossover study in 51 menopausal women (11). After a 1-week assessment period, subjects received either 40mg of red clover isoflavones (Promensil®) or placebo daily. After 12 weeks, all patients received placebo for one month, then crossed over to the alternative arm for a further 14 weeks of treatment. Subjects maintained symptom diaries that were evaluated at the start and end of treatment. Biochemical profiles, complete blood counts, vaginal swabs, and vaginal ultrasound scans were performed, as well as isoflavone levels (determined by 24-hour urine collection). The study found no significant differences in hot flashes, flushing or other physiological parameters between the Promensil® and placebo groups. Treatment was well tolerated, and no adverse effects were reported. The study used a validated self-reporting instrument to measure results. However, there were several methodological weaknesses with this trial: The natural progression of hot flashes and related symptoms is characterized by a waxing and waning progression with time. This trial may have been too short to adequately measure a response; a 1-2 year course would be more appropriate. The study was not investigator-blinded, which may have introduced bias. No power calculation was conducted, and given the small sample size and negative result, it is conceivable that a larger study group may have been necessary to detect important clinical benefits. In addition, many subjects in the placebo groups displayed higher urinary isoflavone levels at the end of the trial compared to baseline, raising questions about possible dietary confounders or misclassification. Due to the crossover design of this study, a carryover effect in the group receiving isoflavones initially may have brought results closer to a null effect.
- A randomized, placebo controlled study by Knight et al. examined menopausal symptoms in 37 postmenopausal women having at least three hot flashes per day (age range 40-65, amenorrheic  $\geq 6$  months) (6). Subjects were given either 40mg or 160mg daily of isoflavones from red clover (Promensil®), or placebo. After 12 weeks of therapy, no statistically significant difference in alleviation of symptoms was observed between the 40mg, 160mg, and placebo groups (reduction in hot flashes 29%, 34%, and 29% respectively). A small but insignificant increase in urinary isoflavones was noted in the placebo group, which may have been due to other dietary phytoestrogen consumption (alfalfa taken by one subject, possibly skewing the mean).
- A randomized, double-blind, placebo controlled trial was reported by Jeri et al. as an abstract only at the 9th International Menopause Society World Congress on the Menopause (1999) (16). In this study, 30 postmenopausal Peruvian women were given 40mg of red clover isoflavones (Promensil®) or placebo for four months. The authors reported significant reductions in hot flash frequency in 73% of red clover patients vs. 20% in placebo, and a 47% reduction in hot flash severity ( $p < 0.001$ ). However, details of blinding, randomization, measurements, and statistical analysis were limited. Without further details provided, the results cannot be adequately analyzed or considered conclusive.

- In an abstract presented at the *North American Menopause Society's 12th Annual Meeting* (2001) (15), and subsequently published in the journal *Maturitas* (34), van de Weijer and Barentsen reported the results of a randomized, double-blind, placebo controlled study of 80mg red clover isoflavones/day (Promensil®) in 30 menopausal women (ages 49-65). After 12 weeks of treatment, the authors reported a 44% decrease in the median frequency of hot flashes vs. no change in placebo, although it is not entirely clear how this calculation was made, and it is not apparent that there was a statistically significant difference between groups. No significant differences between groups were found in the patient-reported "Greene score" of symptom severity. Of some interest, an inverse relationship between urine isoflavone levels and hot flashes was found. This poorly reported study did not provide adequate description of methodology or calculations, had a large dropout (20%), and was not an intention-to-treat analysis (subjects were excluded from analysis after enrollment). Therefore, the results cannot be considered conclusive.
- Case series:** In a case series presented at the *9th International Menopause Society World Congress on the Menopause* (1999), Nachtigall et al. administered 40mg red clover isoflavones/day (Promensil®) to 23 menopausal women (mean age 53), who experienced  $\geq 5$  hot flashes/day and were not taking hormonal therapy (12). Subjects reported their own menopausal symptoms using a 4-point scoring system ("Greene score"). After two months of treatment, a statistically significant 56% reduction in hot flash frequency, 43% reduction in hot flash severity, and 52% reduction in night sweat severity were reported. Transvaginal ultrasound revealed no changes in endometrial thickness. Although these results are suggestive, the design flaws inherent to case series make the results impossible to extrapolate to clinical practice: lack of controls in the setting of a condition which waxes and wanes; lack of blinding which may introduce bias; short duration and small sample size.
- In an abstract presented at the British Menopause Society (2001), Abernathy et al. reported a case series in 33 postmenopausal women (ages 40-65) (35). Subjects received 40mg red clover isoflavones/day (Promensil®) for 12 weeks, at which time a statistically significant 58.5% decrease in frequency of hot flashes was found. A non-significant reduction in the patient-reported "Greene score" of symptom severity was found. Although suggestive, this abstract lacked any description of methods or statistical analysis, and as a case series has the same limitations as described for Nachtigall et al. above.

## Hormone Replacement Therapy (HRT)

- Summary:** High-quality controlled clinical trials supporting the use of red clover isoflavones as an alternative to conventional HRT are lacking. *In vitro* estrogenic responses have been observed with the isoflavones genistein and daidzein, and their respective precursors biochanin A and formononetin, as well as with the steroid-like phytochemical coumestrol. However, human data are limited. It has not been established as to what specific doses would be equivalent to ethinyl estradiol or conjugated equine estrogens (32). Evidence that isoflavones may possess similar benefits to those purported for estrogens (reduction of cardiovascular disease, positive effects on lipid profiles, vascular benefits) has not been demonstrated, and in fact remains controversial for estrogens in general (19;36;37).
- Evidence:** Nestel et al. conducted a randomized, double-blind, controlled study in 26 post-menopausal women (7). After a three-week run-in period in which all subjects received placebo, the women were randomized to placebo or Promensil® (each tablet standardized to 40mg isoflavones derived from red clover). Treatment was divided into three 5-week phases: 40mg or 80mg of isoflavones daily, or

placebo. Dietary education and instruction were provided prior to and throughout treatment. Isoflavone excretion was measured via 24-hour urinary collection. The study found that arterial compliance significantly increased dose-dependently in the isoflavone group compared to placebo. This small study had several methodological flaws. Subjects were inappropriately randomized (every fifth subject was allocated to receive placebo), and complete data were collected only from 60% of treated subjects entered into the study. These design weaknesses make it difficult to draw any conclusions from this trial.

## Osteoporosis

- **Summary:** It is unclear to what extent bone loss is affected by dietary isoflavones such as those present in red clover. Most studies investigating isoflavones and bone metabolism have used soy products, which have a higher concentration of the isoflavones genistein and diadzein than red clover, and contain other potentially active ingredients (saponins, pectins, essential fatty acids). Therefore, at this time there is insufficient evidence to recommend for or against red clover as a treatment for osteoporosis.
- **Evidence:** Clifton-Bligh et al. conducted a case series in 46 post-menopausal women in order to determine the effect of red clover isoflavones on bone and lipid metabolism (5). After a single-blind placebo phase, subjects received 28.5mg, 57mg, or 85.5mg of a red clover isoflavone tablet (Rimostil®) daily for six months. Bone mineral density (BMD) of the proximal radius and ulna significantly increased 4.1% over six months in subjects taking 57mg. Subjects taking 85.5mg had a 3% increase in BMD. Although these results are encouraging, this study was methodologically flawed: there was no control group or dietary log to rule out increases in isoflavone-containing foods. In addition, these changes in BMD were large and rapid, raising the question of concomitant therapy with agents known to be highly active such as bisphosphonates, or inaccurate measurements. These results can be interpreted only as preliminary suggestive evidence.
- In a conference abstract by Atkinson et al., preliminary data from a controlled study giving one year of daily Promensil® (40mg red clover isoflavones) or placebo to 107 pre, peri, and post-menopausal women found no significant overall effect on spine and hip bone mineral density (8). Subgroup analysis suggested that spine bone mineral density decreased less with isoflavones in the pre and peri-menopausal groups, but there was no benefit over placebo for post-menopausal women. Details of randomization, blinding, and statistical analysis were not provided. The authors proposed potential benefit in pre and peri-menopausal women, but in light of the limited reporting in this publication and inconclusive supportive evidence elsewhere, these results cannot be considered definitive.
- In a conference abstract describing a dose escalation study by Baber et al., a red clover isoflavone preparation called P081 prepared by the makers of Promensil® was given to 50 post-menopausal women (11). Subjects were randomized to 25, 50, or 75mg for six months, after which time HDL was noted to increase and LDL to decrease significantly in all groups. However, lack of placebo and incomplete description of methodology or statistical analysis limit the clinical applicability of these results.

## Hypercholesterolemia

- **Summary:** Because estrogens have been reported to decrease low-density lipoproteins (LDL) and increase high-density lipoproteins (HDL), some effort has been undertaken to discern the effects of red clover isoflavones, which appear to possess estrogenic activity, on lipid metabolism. To date, the available evidence of red clover's effects on lipid levels in humans remains inconclusive; although most available research in humans reports no clinically significant effects, due to the small sample sizes used and other methodological weaknesses, a firm conclusion cannot be reached (38;7;36). Notably, soy protein, another source of isoflavones, has been reported to reduce serum lipid levels by up to 10%. However, soy proteins contain higher levels of the isoflavones genistein and diadzein than red clover, and contain other potentially active ingredients (saponins, pectins, essential fatty acids). Preliminary evidence suggests that soy protein is superior to isolated red clover isoflavones for reduction of serum lipid levels.
- **Negative Evidence:** Howes et al. conducted a randomized, controlled, double-blind trial to assess the effect of dietary isoflavone supplementation on lipid levels in 93 post-menopausal women with elevated total cholesterol levels (5.0-9.0 mmol/L) (19). Isoflavones from red clover were extracted to formulate a tablet containing approximately 26mg biochanin A, 16mg formononetin, 0.5mg daidzein, and 1mg genistein. Baseline lipid levels and urine analysis for isoflavones were performed after all subjects received placebo for the first 3-4 weeks. After the run-in period, subjects were randomly assigned to receive placebo or isoflavone therapy, 1 tablet daily for five weeks followed by 2 tablets daily for five weeks. At the end of each treatment period, fasting blood was collected on two consecutive days for lipid analysis, as was a 24-hour urine collection for the measurement of urinary excretion of isoflavones. The trial found no significant changes in total cholesterol, LDL-cholesterol, HDL-cholesterol or plasma triglycerides during supplementation with either dose of isoflavone. Of note, this trial specifically examined the effects of isoflavones derived from red clover vs. soy protein fortified with isoflavones (reported elsewhere to reduce serum lipid levels (39;40)). Soy isoflavones were found to reduce serum lipids, compared with a lack of effect of red clover. However, 18 initial subjects (19.3%) did not complete the trial, and the study design and methods were not well described. Because of the small size and poor methodological quality, this study does not rule out a beneficial effect of red clover isoflavones on lipids.
- Samman et al. conducted a single-blind, randomized controlled trial in 21 healthy women (ages 18-45 years, presumably premenopausal) (17). A red clover isoflavone preparation similar to Promensil® containing 86mg isoflavones/day or placebo was given to subjects for two months prior to crossover. No significant differences were noted in HDL, LDL oxidation, total cholesterol, or triglycerides. However, 33% of subjects dropped out without adequate description of those who left or reasons for leaving, and no power calculation was conducted. It is therefore conceivable that the small sample size and significant attrition rate could have masked potential true benefits of red clover isoflavones. The answer remains inconclusive.
- Blakesmith et al. report the results of a randomized controlled trial in 25 healthy premenopausal women (41). An isoflavone preparation from red clover (86mg/day) was administered to 12 subjects for three menstrual cycles, and placebo was given to the remaining women for four cycles. Although urinary isoflavones increased 15-fold in the red clover group, no significant mean changes were observed in LDL, HDL, insulin, or glucose concentrations compared to the placebo group. Although suggestive, the small sample size raises the question of whether this study was adequately powered to detect between-group differences.
- **Positive evidence:** Clifton-Bligh et al. conducted a study in 46 post-menopausal women in order to determine the effects of red clover isoflavones on lipid and bone metabolism (5). After a single-blind

placebo phase, subjects received 28.5mg, 57mg, or 85.5mg of a red clover isoflavone tablet (Rimostil®) daily for six months. The study found that HDL levels significantly increased, regardless of dose, and apolipoprotein B levels significantly decreased. Although these results are encouraging, this study was methodologically flawed: patients served as their own controls, and there was no dietary log or urine analysis to rule out increases in isoflavone-enriched food intake or increases due to other lifestyle changes. These results can be interpreted only as preliminary, particularly in light of prior negative evidence described above (19;41).

### Benign Prostatic Hypertrophy (BPH)

- **Summary:** There is inconclusive evidence in support of the use of red clover or isoflavones for benign prostatic hypertrophy (BPH). Proposed mechanisms include anti-androgenic properties (24) or estrogenic effects.
- **Evidence:** Gerber presented an abstract at *The Endocrine Society's 82nd Annual Meeting* (2000) which outlined a case series of red clover isoflavones used for BPH symptomatology (42). This study consisted of 29 men who acted as their own historical "controls," reportedly taking either one or two 500mg tablet(s) (Trinovin®), standardized to 40mg of red clover isoflavones per tablet. In this study, 3 months of treatment significantly decreased mean nocturia frequency (29%,  $P < 0.0003$ ), increased urinary flow rates (9.8%,  $p < 0.15$ ), improved quality of life score (15%,  $p < 0.01$ ), and decreased IPSS (22.65%,  $p < 0.002$ ). Prostate specific antigen (PSA) values, blood biochemistry, and hematology were not altered from baseline, and prostate size was not altered. The abstract did not disclose the estimated prostate volumes for the study participants upon enrollment (an important variable in the assessment of disease severity and projected response to therapy). While these results are suggestive, BPH is a chronic condition meriting a long-term study of outcomes, comparison to standard of care, and adequate reporting of methodology. Notably, this study was funded by an unrestricted educational grant from Novogen, Inc., the makers of Trinovin®.

### Prostate Cancer

- **Summary:** Red clover contains isoflavones, a class of phytoestrogen. Certain isoflavones have been shown *in vitro* to possess antineoplastic properties related to the suppression of angiogenesis, inhibition of tyrosine kinase activity, cellular antiproliferation, anti-androgen effects (24), or partial antagonism of estrogen receptors (28;29;20). The effects of isoflavones have been examined in human cancer cell lines, including prostate and breast cancer cells (43;44;45;46). However, there is no convincing clinical evidence that red clover exerts similar effects in humans.
- **Evidence:** A patient scheduled for radical suprapubic prostatectomy took four 40mg red clover isoflavone tablets (Promensil®) for seven days before surgery. After excision, histologic changes analyzed in the prostatectomy specimen, particularly apoptosis, were interpreted as suggestive of androgen deprivation (18). As a case report, this study is suggestive, but is not a basis for therapeutic recommendation.

## Brands used in statistically significant clinical trials:

- Promensil® (Novogen, labs: Sydney, Australia); Rimostil® (Novogen labs: Sydney, Australia); Trinovin® (Novogen labs: Sydney, Australia).

---

## AUTHOR INFORMATION

- **Authors/Editors:** Jamie Nelsen, PharmD (University of Rhode Island); Catherine Ulbricht, PharmD (Massachusetts General Hospital); Ernie-Paul Barrette, MD (Case Western Reserve School of Medicine); Edzard Ernst, MD, PhD (University of Exeter); Philippe Szapary, MD, MPH (University of Pennsylvania); David Sollars MAc, HMC (New England School of Acupuncture); Candy Tsourounis, Pharm D (University of California, San Francisco); Adrienne Rogers, MD (Boston University School of Medicine); Samuel Basch, MD (Mt. Sinai Medical Center, NY); Sadaf Hashmi, MD, MPH (Johns Hopkins School of Hygiene and Public Health); Ethan Basch, MD (Memorial Sloan-Kettering Cancer Center); Steve Bent, MD (University of California, San Francisco).
- **Blinded Peer Review:** Natural Standard Editorial Board

---

## REFERENCES

1. Nelsen J, Ulbricht C, Barrette EP, et al. Red clover (*Trifolium pratense*) monograph: a clinical decision support tool. *Journal of Herbal Pharmacotherapy* 2002;2(3):49-72.
2. Campbell MJ, Woodside JV, Honour JW, et al. Effect of red clover-derived isoflavone supplementation on insulin-like growth factor, lipid and antioxidant status in healthy female volunteers: a pilot study. *Eur J Clin Nutr* 2004;58(1):173-179. [View Abstract](#)
3. Howes JB, Tran D, Brillante D, et al. Effects of dietary supplementation with isoflavones from red clover on ambulatory blood pressure and endothelial function in postmenopausal type 2 diabetes. *Diabetes Obes Metab* 2003;5(5):325-332. [View Abstract](#)
4. Ingram D, Sanders K, Kolybaba M, et al. Case-control study of phyto-oestrogens and breast cancer. *Lancet* 1997;350(9083):990-994. [View Abstract](#)
5. Clifton-Bligh PB, Baber RJ, Fulcher GR, et al. The effect of isoflavones extracted from red clover (Rimostil) on lipid and bone metabolism. *Menopause* 2001;8(4):259-265. [View Abstract](#)
6. Knight DC. The effect of Promensil, an isoflavone extract, on menopausal symptoms. *Climacteric* 1999;2:79-84.
7. Nestel PJ, Pomeroy S, Kay S, et al. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. *J Clin Endocrinol Metab* 1999;84(3):895-898. [View Abstract](#)
8. Atkinson C, Compston JE, Robins SP, et al. The effects of isoflavone phytoestrogens on bone: preliminary results from a large randomized controlled trial. *Endocr Soc Annu Meet Program* 2000;82:196.
9. Atkinson C, Warren RM, Dowsett M, et al. Effects of isoflavones on breast density, oestradiol, and gonadotrophins: A double blind randomised placebo controlled trial. Poster presented at: The Endocrine Society's 83rd Annual Meeting (2000).
10. Zava DT, Dollbaum CM, Blen M. Estrogen and progestin bioactivity of foods, herbs, and spices. *Proc Soc Exp Biol Med* 1998;217(3):369-378. [View Abstract](#)

11. Baber R, Clifton Bligh P, Fulcher G, et al. The effect of an isoflavone extract (PO81) on serum lipids, forearm bone density and endometrial thickness in postmenopausal women [Abstract]. Proceedings of the North American Menopause Society (New York, 1999).
12. Nachtigall LB, La Grega L, Lee WW, et al. The effects of isoflavones derived from red clover on vasomotor symptoms and endometrial thickness. 9th International Menopause Society World Congress on the Menopause. 1999;331-336.
13. Krenn L, Unterrieder I, Rupprechter R. Quantification of isoflavones in red clover by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002;777(1-2):123-128. [View Abstract](#)
14. LeRoy A, Potter E, Woo HH, et al. Characterization and identification of alfalfa and red clover dietary supplements using a PCR-based method. *J Agric Food Chem* 2002;50(18):5063-5069. [View Abstract](#)
15. van de Weijer, Barentsen R. Isoflavones from red clover (Promensil®) significantly reduce hot flashes compared with placebo. Poster presented at: The North American Menopause Society's 12th Annual Meeting (2001).
16. Jeri AR. The effect of isoflavone phytoestrogens in relieving hot flushes in Peruvian post menopausal women. 9th International Menopause Society World Congress on the Menopause (1999).
17. Samman S, Lyons Wall PM, Chan GS, et al. The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. *Atherosclerosis* 1999;147(2):277-283. [View Abstract](#)
18. Stephens FO. Phytoestrogens and prostate cancer: possible preventive role. *Med J Aust* 1997;167(3):138-140. [View Abstract](#)
19. Howes JB, Sullivan D, Lai N, et al. The effects of dietary supplementation with isoflavones from red clover on the lipoprotein profiles of post menopausal women with mild to moderate hypercholesterolaemia. *Atherosclerosis* 2000;152(1):143-147. [View Abstract](#)
20. Umland EM, Cauffield JS, Kirk JK, et al. Phytoestrogens as therapeutic alternatives to traditional hormone replacement in postmenopausal women. *Pharmacotherapy* 2000;20(8):981-990. [View Abstract](#)
21. Messina M, Barnes S, Setchell KD. Phyto-oestrogens and breast cancer. *Lancet* 1997;350(9083):971-972. [View Abstract](#)
22. Hilakivi-Clarke L, Cho E, Onojafe I, et al. Maternal exposure to genistein during pregnancy increases carcinogen- induced mammary tumorigenesis in female rat offspring. *Oncol Rep* 1999;6(5):1089-1095. [View Abstract](#)
23. Zand RS, Jenkins DJ, Diamandis EP. Steroid hormone activity of flavonoids and related compounds. *Breast Cancer Res Treat* 2000;62(1):35-49. [View Abstract](#)
24. Jarred RA, McPherson SJ, Jones ME, et al. Anti-androgenic action by red clover-derived dietary isoflavones reduces non-malignant prostate enlargement in aromatase knockout (ArKo) mice. *Prostate* 2003;56(1):54-64. [View Abstract](#)
25. Liu J, Burdette JE, Xu H, et al. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem* 2001;49(5):2472-2479. [View Abstract](#)
26. Burdette JE, Liu J, Lantvit D, et al. *Trifolium pratense* (red clover) exhibits estrogenic effects in vivo in ovariectomized Sprague-Dawley rats. *J Nutr* 2002;132(1):27-30. [View Abstract](#)
27. Jarred RA, Keikha M, Dowling C, et al. Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones. *Cancer Epidemiol Biomarkers Prev* 2002;11(12):1689-1696. [View Abstract](#)
28. Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. *Obstet Gynecol* 1996;87(5 Pt 2):897-904. [View Abstract](#)
29. Yanagihara K, Ito A, Toge T, et al. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res* 1993;53(23):5815-5821. [View Abstract](#)
30. Barnes S, Kim H, Darley-Usmar V, et al. Beyond ERalpha and ERbeta: estrogen receptor binding is only part of the isoflavone story. *J Nutr* 2000;130(3):656S-657S. [View Abstract](#)
31. Domon OE, McGarrity LJ, Bishop M, et al. Evaluation of the genotoxicity of the phytoestrogen, coumestrol, in AHH- 1 TK(+/-) human lymphoblastoid cells. *Mutat Res* 2001;474(1-2):129-137. [View Abstract](#)
32. Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. *J Nutr* 1999;129(3):758S-767S. [View Abstract](#)
33. Setchell KD. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* 1998;68(6 Suppl):1333S-1346S. [View Abstract](#)
34. van de Weijer PH, Barentsen R. Isoflavones from red clover (Promensil) significantly reduce menopausal hot flush symptoms compared with placebo. *Maturitas* 2002;42(3):187-193. [View Abstract](#)
35. Abernethy K, Brockie J, Suffling K, et al. An open study of the effects of a 40mg isoflavone food supplement (derived from Red Clover), on menopausal symptoms. The British Menopause Society (2001).
36. Simons LA, von Konigsmark M, Simons J, et al. Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. *Am J Cardiol* 2000;85(11):1297-1301. [View Abstract](#)
37. Finking G, Wohlfrom M, Lenz C, et al. The phytoestrogens Genistein and Daidzein, and 17 beta-estradiol inhibit development of neointima in aortas from male and female rabbits in vitro after injury. *Coron Artery Dis* 1999;10(8):607-615. [View Abstract](#)

38. Hodgson JM, Puddey IB, Beilin LJ, et al. Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. *J Nutr* 1998;128(4):728-732. [View Abstract](#)
39. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 1995;333(5):276-282. [View Abstract](#)
40. Lovati MR, Manzoni C, Canavesi A, et al. Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. *J Clin Invest* 1987;80(5):1498-1502. [View Abstract](#)
41. Blakesmith SJ, Lyons-Wall PM, George C, et al. Effects of supplementation with purified red clover (*Trifolium pratense*) isoflavones on plasma lipids and insulin resistance in healthy premenopausal women. *Br J Nutr* 2003;89(4):467-474. [View Abstract](#)
42. Gerber G, Lowe FC, Spigekman S. The use of a standardized extract of red clover isoflavones for the alleviation of BPH symptoms. *The Endocrine Society's 82nd Annual Meeting* 2000;82:2359.
43. Le Bail JC, Champavier Y, Chulia AJ, et al. Effects of phytoestrogens on aromatase, 3beta and 17beta-hydroxysteroid dehydrogenase activities and human breast cancer cells. *Life Sci* 2000;66(14):1281-1291. [View Abstract](#)
44. Peterson G, Barnes S. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate* 1993;22(4):335-345. [View Abstract](#)
45. Peterson G, Barnes S. Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. *Biochem Biophys Res Commun* 1991;179(1):661-667. [View Abstract](#)
46. Barnes S. Effect of genistein on in vitro and in vivo models of cancer. *J Nutr* 1995;125(3 Suppl):777S-783S. [View Abstract](#)