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Clinical studies of red clover (*Trifolium pratense*) dietary supplements in menopause: a literature review

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ABSTRACT

Red clover (*Trifolium pratense* L., Fabaceae) botanical dietary supplements have received much attention recently for their potential use in the treatment of menopause symptoms, maintenance/improvement of bone and cardiovascular health, and reported benign effects on the breast and endometrium. Literature searches of four computerized databases were run to identify clinical studies of red clover botanical dietary supplements. The manufacturer of the red clover products used in the majority of the studies was contacted for unpublished information and/or clarification regarding the chemical content of their products. Red clover studies were reviewed that pertained to women's health or menopause. Clinical evidence is presently lacking to support the efficacy of semipurified red clover isoflavone extracts for alleviation of climacteric vasomotor symptoms or reduction of low-density lipoprotein levels in the blood. Furthermore, the safety of use of red clover isoflavone supplements in patients with breast or endometrial cancer has not been established. Limited evidence suggests possible efficacy in maintenance of bone health and improvement of arterial compliance, a risk factor for atherosclerosis. This literature review covers red clover botanical dietary supplement clinical studies having a possible impact on the health care of mature and menopausal women, and provides historical perspective regarding the traditional uses of red clover.

Key Words: *Trifolium pratense* – Red clover – Menopause – Cardiovascular health – Bone health – Botanical dietary supplement.

Over the last several years, semipurified isoflavone supplements made from red clover have been studied for use in menopause,[1-7] maintenance of bone health,[8-12] and improvement of cardiovascular health.[13,14] Evidence for efficacy in preventing loss of mineral density in specific bones is promising,[8,9,11] although a consensus has not yet been reached. Several studies indicate that red clover isoflavone supplements improve arterial compliance,[13,14] a risk factor for atherosclerosis, but more data are needed. Trials examining the ability of red clover isoflavone

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supplements to alleviate menopausal hot flashes[1-7] and lower serum lipids[2,9,10,12,13,15-21] (cholesterol, triglycerides) have not shown promise overall, although study methodologies and/or the patients' baseline levels of lipoproteins in the studies may have been complicating factors.

Red clover was traditionally valued mainly as a respiratory antispasmodic[22-24] and an anticancer treatment,[25] and not so much as an estrogenic agent. However, observations of and subsequent research into the estrogenic effects of *Trifolium* species on grazing[26-30] and experimental livestock[31,32] brought to light the relevance of red clover isoflavones. Translation of the findings in animals has stimulated the recent development and sale of red clover botanical dietary supplements for hormonally related conditions in humans.

Red clover contains several general classes of compounds but is particularly rich in isoflavones, flavones, and flavonols. Soy (*Glycine max* L., Fabaceae) and red clover both contain the isoflavones daidzein (**1**) and genistein (**2**), and soy may contain small amounts of formononetin (**3**) and biochanin A (**4**). (See Fig. 1 for chemical structures.) Red clover, however, contains substantially more formononetin and biochanin A, relative to genistein and daidzein, when compared with soy. Accordingly, isoflavone supplements produced from soy and red clover will not have the same levels of individual isoflavones, even if the "total isoflavone" content is comparable. Some researchers do not appreciate the distinct chemical profiles; and by extension, comparisons of trials of soy and red clover isoflavone products may not be valid. Commercial red clover isoflavone supplements are semipurified and do not contain a protein fraction, whereas many soy products do. In short, although there have been numerous clinical publications on the study of "soy isoflavones" and "red clover isoflavones," it is now necessary to better define the chemical content of red clover and soy supplements beyond "total isoflavones" in order that correlation and dose-response studies for individual molecules may be performed. Effort was made in this review to describe known attempts at independent verification of product contents when provided by the study authors.

HISTORICAL CONTEXT OF RED CLOVER USE

External uses

Red clover blossoms have been incorporated into ointments or decocted (boiled in water) to make compresses for "ulcers,"[33-35] believed to be cancerous lesions or growths by other authors.[25] These preparations were also used to treat burns,[33] wounds,[36,37] gout,[38] and fungal infections.[33] The expressed juice has been used for eye diseases.[23]

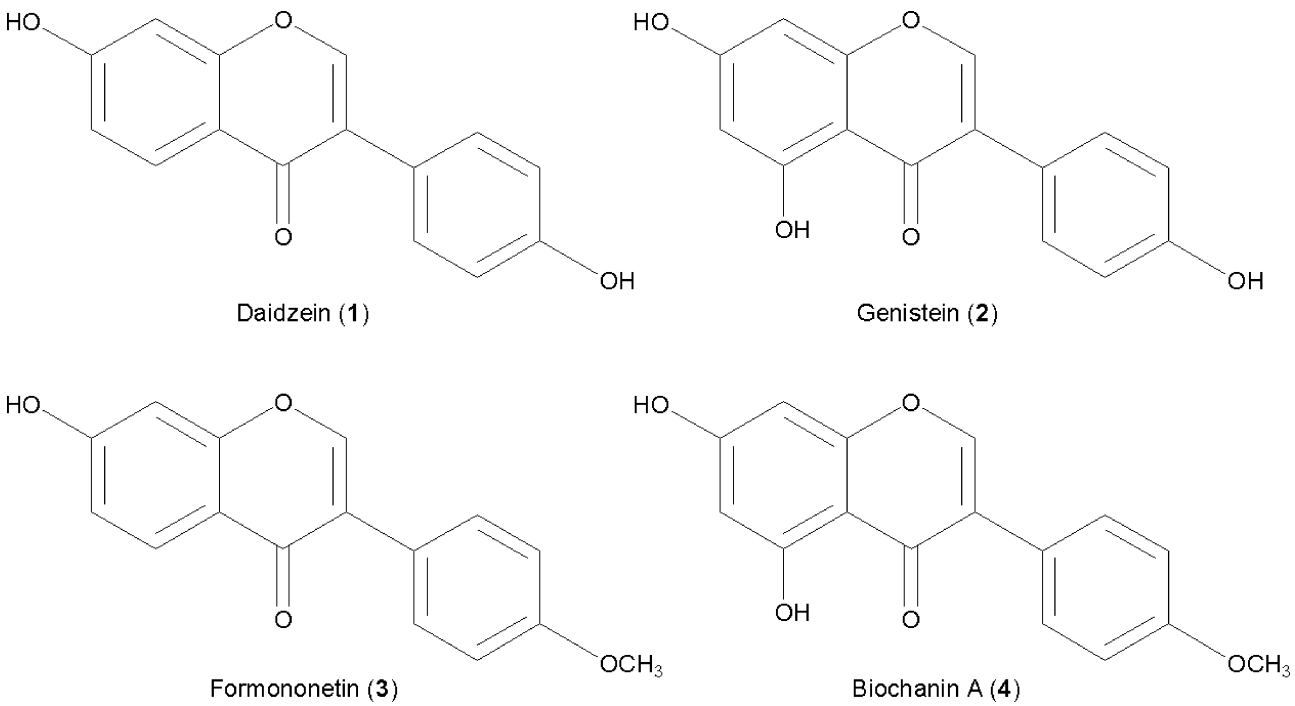

FIG. 1. Structures of the four main isoflavones found in red clover.

Internal uses

Cherokee Indians used a tea of the flowers or aboveground parts to treat fevers, "Bright's disease" (nephritis), and leukorrhea.[39] The Iroquois referred to red clover as a "blood medicine."[40] The Ute of Utah and Nevada used a decoction as an abortifacient.[41] Red clover tea or tincture (ethanolic extract) was used as an antispasmodic in whooping cough, measles, bronchitis, laryngitis, and tuberculosis in the 19th and 20th centuries.[42] Red clover cigarettes were considered a treatment for asthma, according to an early edition of the *National Formulary*.[43] Red clover decoctions (extraction by continuous boiling in water) and infusions (steeped in cool or hot water, without boiling) are still used as expectorants,[34] alteratives,[44] sedatives,[33,45] and remedies for rheumatism.[33] Less frequently, red clover is used for inducing menstruation,[46] aiding change of life in women,[40] or as a fertility tonic.[45] It should be noted that the efficacies of most of the abovementioned traditional uses of red clover have not yet been tested in placebo-controlled, randomized clinical studies.

Commercial products

Commercial internal remedies containing red clover have been marketed for numerous conditions. In 1900, a product named "Extract of Trifolium Compound," produced by the Wm. S. Merrell Chemical Company (Cincinnati, OH), contained potassium iodide plus extracts of the following plants: *T pratense* L. (red clover), *Stillingia sylvatica* L. (queen's root), *Lappa minor* Hill (also known as *Arctium minus* (Hill) Bernh.; common or lesser burdock), *Phytolacca decandra* L. (poke root), *Picramnia antidesma* S. W. (cascara amarga), *Berberis aquifolium* Pursh (Oregon grape), *Podophyllum peltatum* L. (May apple), and *Xanthoxylum carolinianum* Lamarck (Southern prickly ash).[42] This preparation was recommended for treatment of syphilis, scrofula, chronic rheumatism, and various skin afflictions. The formula for the controversial Hoxsey internal cancer remedy has likely changed over time and been customized for individual patients; however, it has probably contained, at one time or another, the following plants (plus potassium iodide): *Phytolacca americana* L. (poke weed), *Arctium lappa* L. (also known as *Lappa major* Gaertn.; edible or great burdock), *Berberis vulgaris* L. (barberry), *Rhamnus frangula* L. (buckthorn), *Stillingia sylvatica* L. (queen's root), *Zanthoxylum americanum* Mill. (Northern prickly ash), *Rhamnus purshiana* De Candolle (cascara sagrada) and/or *Picramnia antidesma* S. W. (cascara amarga), *Glycyrrhiza glabra* L. (licorice), *Medicago sativa* L. (alfalfa) and *T pratense* L. (red clover).[47] Flor-Essence, manufactured by Flora, Inc. (Lynden, WA) and Flora Manufacturing & Distributing, Ltd. (Burnaby, British Columbia, Canada), is sometimes used by cancer patients and contains the following plant extracts: *Arctium lappa* L., *T pratense* L., *Cnicus benedictus* L. (blessed thistle), *Ulmus rubra* Muhl. and/or *Ulmus fulva* Muhl. (slippery elm), *Rumex acetosella* L. (sheep sorrel), *Rheum palmatum* L. (Turkish rhubarb), *Lami-naria digitata* Lmx. (kelp), and *Nasturtium officinale* R. Br. (watercress). Numerous other red clover botanical supplements are currently sold in North America, the most widely distributed being Promensil, Rimostil, and Trinovin, all manufactured by Novogen, Ltd. (North Ryde NSW, Australia). Available clinical evidence on red clover, described herein, has largely been collected on these three products.

CLINICAL STUDIES OF RED CLOVER

METHODS

Identification of Clinical Trials

Computerized literature searches were systematically performed to identify any clinical studies of red clover botanical dietary supplements pertinent to women's health and menopause. The databases used were PubMed, SciFinder Scholar, Web of Science (Science Citation Index Expanded), and NAPRALERT (NAtural PRoducts ALERT database, University of Illinois at Chicago, Chicago, IL). The main search term used was red clover and a subsearch was done using the term clinical to help discern scientific studies from reviews. Individual results were evaluated for pertinence to women's health or menopause. Novogen, the manufacturer of the red clover products Promensil, Trinovin, and Rimostil, was contacted and asked to provide information on the chemical content of their commercial and experimental products (Table 1), and to verify which products were tested in certain studies.

Inclusion and exclusion criteria

Clinical studies of red clover botanical dietary supplements were considered that contained outcome measures (eg, physical, psychological, biochemical) relating to the following topic areas: 1) menopause, 2) cognitive effects, 3) bone health, 4) cardiovascular health (including both vascular and lipid effects), 5) breast effects, 6) endometrial effects, 7) insulin effects, and 8) antioxidant effects. Although no human studies were available under the topic headings of

TABLE 1. *Reported isoflavone content of red clover products used in clinical studies*

Product or formulation and manufacturer	Manufacturer reported (G+B):(D+F) ratio	Milligrams per tablet				Total isoflavones per tablet	References
		D	G	F	B		
Promensil Novogen, Ltd., North Ryde NSW, Australia (40 mg total isoflavones)	1.9 : 1	0.5	1	16	26	43.5 mg	Atkinson et al[7,11,21] (2004a, 2004b, 2004c), Howes et al[16] (2000)
		5	4	8	25	42 mg	Schult et al[12] (2004), Campbell et al[19] (2004)
		3.5	4	8	24.5	40 mg	Knight et al[2] (1999), Nestel et al[13] (1999)
Rimostil Novogen, Ltd., North Ryde NSW, Australia (57 mg total isoflavones)	0.15 : 1	<1	<1	25	2.5	< 29.5 mg	Schult et al[12] (2004), Howes et al[18] (2003), Howes et al[48] (2004)
Trinovin Novogen, Ltd., North Ryde NSW, Australia (40 mg total isoflavones)	1.9 : 1	N.R.				N.R.	N.R.
P-07 Novogen, Ltd., North Ryde NSW, Australia (40 mg total isoflavones)	3.8 : 1	3.7	4.3	9.3	25.7	42 mg/tab	Blakesmith et al[17] (2003)
P07(b) Novogen, Ltd., North Ryde NSW, Australia (40 mg total isoflavones)	3.5 : 1	N.R.				N.R.	N.R.
P-083 Novogen, Ltd., North Ryde NSW, Australia (40 mg total isoflavones)	0.2 : 1	N.R.				N.R.	N.R.
Phytogyn Gynea, Barcelona, Spain (38 mg red clover isoflavones; 17 mg soy isoflavones)	N/A	N.R.				N.R.	Garcia-Martinez et al[57] (2003)

D, daidzein; G, genistein; F, formononetin; B, biochanin A; N.R., no independent reports of chemical analysis were found.

thyroid effects and chemotherapeutic/chemopreventive effects, limited data from animal and/or in vitro studies were included in the interest of presenting a well-rounded view of the possible physiological effects of red clover. Studies involving soy or soy isoflavones supplements were not included in this review.

Data Extraction and Evaluation

The data were extracted and summarized in tabular form (see Tables 2 through 6) according to the study type and design, ie, double-blind, placebo-controlled, randomized, etc. Both primary and secondary endpoint data (physical, psychological, and biochemical) were summarized with some studies appearing more than once, under multiple topic headings. No quantitative or statistical analyses of the information were attempted, although qualitative judgments are presented regarding some issues such as study quality and length of placebo run-in period. Figure 2 contains a general overview of the number of clinical studies under each topic heading.

Menopause

Studies that have administered semipurified red clover preparations to women to relieve menopausal hot flashes (Table 2) have generally been of short

TABLE 2. *Clinical studies of red clover for menopausal symptoms*

Reference	Product(s) tested	Dose	Study design	Outcome
Baber et al[1] 1999	Promensil	40 mg isoflavones	DB, PC, R, cross-over, 3 mo, 51 women	No change in hot flashes
Knight et al[2] 1999	Promensil	40/160 mg isoflavones	DB, PC, R, 3 mo, 37 women	No change in hot flashes
Nachtigall et al[4] 1999	Promensil	40 mg isoflavones	Uncontrolled, 2 mo, 23 women	56% decrease in hot flashes
van de Weijer et al[5] 2002	Promensil	80 mg isoflavones	DB, PC, R, 3 mo, 30 women	44% decrease in hot flashes
Jeri et al[3] 2002	Promensil	40 mg isoflavones	DB, PC, R, 4 mo, 30 women	48% decrease in hot flashes
Tice et al[6] 2003	Promensil; Rimostil	80/57 mg isoflavones	DB, PC, R, 3 mo, 252 women	No change in hot flashes
Atkinson et al[7] 2004a	Promensil	43.5 mg isoflavones	DB, PC, R, 1 y, 177 women	No change in hot flashes

DB, double-blind; PC, placebo-controlled; R, randomized.

TABLE 3. *Clinical studies of red clover for bone health*

Reference	Product(s) tested	Dose	Study design	Outcome
Kelly and Husband[8] 2000 (patent)	Not stated; 15:1 to 2:1 ratio of formononetin to (daidzein + genistein + biochanin A)	25, 50, or 75 mg of isoflavones	50 postmenopausal women, 6 mo; SB; 1 mo placebo run-in	2.9, 4.1, and 3.0% increase in proximal forearm BMD, respectively; no change in distal forearm BMD
Clifton-Bligh et al[9] 2001	Rimostil	28.5, 57, or 85.5 mg isoflavones	DB, R, SB placebo phase, 46 perimenopausal women, 6 mo	Mid- and high-dose groups had 4.1% and 3.0% increase in BMD of proximal radius and ulna; no change low-dose group
Hale et al[10] 2001	P-07	50 mg isoflavones	DB, PC, R, 30 pre- and perimenopausal women, 3 mo	No change in *N*-telopeptide or osteocalcin bone marker levels
Atkinson et al[11] 2004b	Promensil	43.5 mg isoflavones	DB, PC, R, 205 (177 after dropout) pre-, peri-, and postmenopausal women, 1 y	Decreased loss of BMC and BMD in lumbar spine in treatment group; no change in hip BMD or bone resorption markers
Schult et al[12] 2004	Promensil; Rimostil	82 or 57.2 mg isoflavones	DB, PC, R, 252 (245 after dropout) peri- and menopausal women, 3 mo	No change (either treatment) in urinary *N*-telopeptide or serum osteocalcin levels

SB, single-blind; BMD, bone mineral density; DB, double-blind; PC, placebo-controlled; R, randomized; BMC, bone mineral content.

duration (≤12 weeks) and any positive results, when achieved, usually take 8 weeks to manifest. Studies generally demonstrate a significant placebo effect during the first 4 weeks that may persist throughout an investigation. More recent studies have incorporated a 2- or 4-week run-in period to assess this placebo effect. Evidence for red clover efficacy in reduction of hot flashes is not compelling; nor have any studies directly compared red clover to a pharmaceutical entity proven to alleviate hot flashes (positive control).

Six short-term trials (≤ 4 months) have investigated red clover isoflavone supplements for efficacy in relief of hot flashes, and only three of these have yielded positive results. These three studies were all published in 1999, and used the product Promensil. A 2-month study in 23 menopausal women[4] (aged 40-65 years) administered one Promensil tablet daily and reported a 56% reduction in frequency of hot flashes and a 43% decrease in intensity. However, this was an uncontrolled study, so any contribution from the placebo effect was not determined. The second study[1] was a double-blind, randomized, placebo-controlled 3-month trial in 51 postmenopausal women (aged 45-65 years), which began with a 1-week run-in period. One tablet of Promensil was given daily, and no reduction in frequency of hot flashes was noted for the treatment group. The third study[2] administered placebo or one or four tablets of Promensil daily to 37 postmenopausal women (aged 40-65 years) over 3 months. The authors noted a large placebo response and no difference between treatment groups versus placebo.

Two menopause studies of red clover isoflavones were published in 2002. The first was a 4-month, randomized, double-blind study[3] that evaluated 30 postmenopausal Peruvian women (younger than 60 years; median age 52 ± 0.7 years) who were given either one tablet of Promensil or placebo daily. Frequencies of hot flushes decreased 49% and 11%,

TABLE 4. *Clinical studies of red clover for vascular effects*

Reference	Product(s) tested	Dose	Study design	Outcome
Nestel et al[13] 1999	Promensil	40 mg, then 80 mg isoflavones	14, 13, 3 (constant placebo) menopausal women, 8 wk run-in/placebo, 2 × 5 wk subsequent treatment	Significant increase in arterial compliance, both treatment groups
Baber et al[1] 1999	Rimostil	28.5, 57, or 85.5 mg isoflavones	R, uncontrolled, 50 postmenopausal women, 6 mo	No change in serum factor V, VII, VIII, antithrombin III, or fibrinogen levels
Teede et al[14] 2003	P-07(b) (Biochanin A-enriched); P-083 (Formononetin-enriched)	80 mg isoflavones	DB, R, normotensive subjects, 46 men, 34 postmenopausal women, 2 × 6 wk treatment, with crossover and 1 wk washout in-between	Formononetin-enriched P-083 significantly increased arterial compliance and decreased VCAM-1 plasma levels

DB, double-blind; PC, placebo-controlled; R, randomized; VCAM-1, vascular cellular adhesion molecule-1.

TABLE 5. *Red clover clinical studies measuring lipid parameters*

Reference	Product(s) or formulation(s) tested	Dose	Study design	Outcome
Nestel et al[13] 1999	Promensil	40, then 80 mg of isoflavones	14, 13, 3 (constant placebo) menopausal women, 2 × 5 wk subsequent treatment, 8 wk run-in/placebo	No change in plasma lipids
Baber et al[15] 1999b	Rimostil	28.5, 57, or 85.5 mg of isoflavones	R, uncontrolled, 50 postmenopausal women, 6 mo	Increase in HDL, all groups; decrease in apolipoprotein B (ApoB)
Knight et al[2] 1999	Promensil	40/160 mg of isoflavones	DB, PC, R, 37 postmenopausal women, 3 mo	Increase in HDL, 40 mg group
Nachtigall et al[4] 1999	Promensil	40 mg of isoflavones	Uncontrolled, 23 women, 2 mo	No change in plasma lipids
Kelly and Husband[8] 2000	Novogen, Ltd. formulation (unspecified; 15:1 to 2:1 ratio of formononetin to (daidzein + genistein + biochanin A)	25, 50, 75 mg of isoflavones	SB, 1 mo placebo run-in, 50 postmenopausal women, 6 mo	Increase in HDL and decrease in ApoB (all treatments)
Howes et al[16] 2000	Promensil	40/80 mg of isoflavones	DB, R, 93 menopausal women (75 after dropout), 9 (constant placebo), 4 wk/dose	No change in plasma lipids
Clifton-Bligh et al[9] 2001	Rimostil	28.5, 57, or 85.5 mg of isoflavones	DB, R, SB placebo phase, 46 perimenopausal women, 6 mo	Increase in HDL, decrease in ApoB
Hale et al[10] 2001	P-07	50 mg of isoflavones	DB, R, PC, 30 pre-/ perimenopausal women, 3 mo	No change in plasma lipids
Garcia-Martinez et al[57] 2003	Phytogyn	38 mg of red clover isoflavones plus 17 mg of soy isoflavones	Uncontrolled, 25 postmenopausal women, 6 mo	Significant decrease in TGs
Blakesmith et al[17] 2003	P-07	86 mg of isoflavones	DB, R, PC, parallel, 25 premenopausal women, 3 menstrual cycles	No change in plasma lipids
Howes et al[18] 2003	Rimostil? (not specified, but chemical content matches)	57 mg of isoflavones	DB, PC, R, crossover, 16 postmenopausal type 2 diabetics, 4 wk	No change in plasma lipids
Campbell et al[19] 2004	Promensil	86 mg of isoflavones	PC, R, crossover, 16 pre- and 7 postmenopausal women, 1 mo	Increase in HDL in postmenopausal women only
Schult et al[12] 2004	Promensil, Rimostil	82 or 57.2 mg of isoflavones	DB, PC, R, 252 (247 after dropout) peri- and menopausal women, 3 mo	Decrease in TGs (both treatments) for women having ≥ 178 mg/dL at baseline
Nestel et al[20] 2004	P-07(b) (Biochanin A-enriched), P-083 (Formononetin-enriched)	40 mg of isoflavones	DB, R, placebo crossover, 46 men, 34 postmenopausal women, 6 wk	P-07(b) decreased LDL by 9.5% in men, no change for women
Atkinson et al[21] 2004c	Promensil	43.5 mg of isoflavones	DB, PC, R, 205 (177 after dropout) pre-, peri- and postmenopausal women, 1 y	Decrease in TGs in perimenopausal women only

DB, double-blind; SB, single-blind; PC, placebo-controlled; R, randomized; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TGs, triglycerides.

respectively, in treatment versus placebo groups and severity of hot flushes decreased 47% versus 0%. A double-blind, randomized placebo-controlled study in 30 Dutch postmenopausal women[5] (aged 49-65 years) indicated that two tablets of Promensil (80 mg of isoflavones/day) reduced the frequency of hot flashes by 44% compared with placebo after 12 weeks, although the mean body mass index (BMI) values for the treatment group were slightly higher (statistically nonsignificant), compared with the placebo group. This study suggests possible effects of isoflavones on metabolism, insulin, and/or changes in stores of hormones in fat depots.

Tice and co-workers published a double-blind, randomized placebo-controlled study[6] in 2003 that demonstrated similar percentage reductions in hot flashes at the end of 12 weeks for two treatment arms (Promensil, 82 mg isoflavones/day, high genistein + biochanin A; Rimostil, 57 mg isoflavones/day, high daidzein + formononetin) and the placebo arm. (For

TABLE 6. *Clinical studies of red clover for breast and endometrial effects*

Reference	Product(s) tested	Dose	Study design	Outcome
Ingram et al[64] 2002	Promensil	40/80 mg of isoflavones	DB, PC, R, 18 women, 3 menstrual cycles, cyclical mastalgia	Significant decrease in breast pain in 40-mg group vs placebo
Atkinson et al[7] 2004a	Promensil	40 mg of isoflavones	DB, PC, R, 177 women at high-risk for breast cancer, 1 y	No change in breast density
Campbell et al[19] 2004	Promensil	86 mg of isoflavones	R, PC, crossover, 16 pre- and 7 postmenopausal women, 1 mo	Nonsignificant decrease in insulin-like growth factor-1 levels in premenopausal women only
Hale et al[10] 2001	P-07	50 mg of isoflavones	DB, PC, R, 30 pre- and perimenopausal women, endometrial proliferation measurement, 3 mo	No change in Ki-67 antigen levels or uterine Doppler resistance
Clifton-Bligh et al[9] 2001	Promensil	28.5, 57, or 85.5 mg of isoflavones	DB, R, uncontrolled, SB placebo phase, 46 postmenopausal women, 6 mo	No change in endometrial thickness

the composition of the various red clover preparations used clinically, see Table 1.) Subjects were aged 45 to 60 years. Although overall results were negative (statistically nonsignificant), both treatment groups showed a slightly higher response in women with a BMI greater than 25.1, further supporting the idea that red clover isoflavones have metabolic effects.

The only published long-term red clover study to date administered one tablet of Promensil daily for 1 year to 117 women (aged 49-65 years) and found no statistically significant changes in mean number of hot flashes or other menopausal symptoms compared with placebo.[7] It is noteworthy that the trial used a low dose of 40 mg total isoflavones. This dose may not have been high enough to be effective.

It should be kept in mind that patterns of phytoestrogen intake in the background diet of patients may impact the outcome of menopause trials. Diet is often influenced by racial, ethnic, or socioeconomic background. The earliest menopause studies of red clover supplements did not survey or control for the isoflavone content in the patients' diets. Although later studies have excluded subjects on isoflavone supplementation or high soy diets, control over intake of other isoflavone-containing foods (ie, chickpeas, red clover, or alfalfa sprouts) has been variable. Methodological differences in patient recruitment and eligibility, most notably different eligibility criteria for levels of follicle-stimulating hormone (FSH), have hampered trial comparisons. Additional long-term menopause studies using higher doses of red clover isoflavones are warranted.

Cognitive effects

Only one published study has examined the effect of red clover isoflavones on cognitive function in postmenopausal women.[48] Thirty women older than 60 years received two tablets of Rimostil or placebo for 6 months. The isoflavone group experienced better results in visual-spatial intelligence testing than the placebo group (12% increase vs 3% decrease, respectively). However, the treatment group showed no improvement in verbal memory (1% increase vs 29% increase, isoflavones vs placebo), and a decline in digit recall (6% decrease vs 12% increase) compared with placebo. The results were

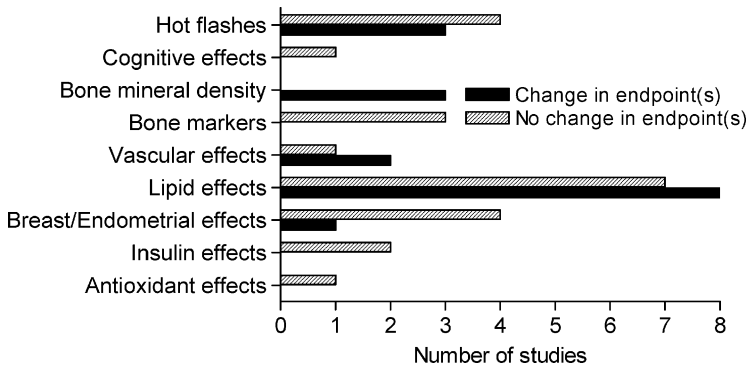

FIG. 2. Overview of the number of clinical studies of red clover supplements and their outcomes according to topic area.

not statistically significant after adjustment for potential chance results due to multiple comparisons, indicating that further trials are required to determine whether red clover isoflavones can significantly affect cognitive function in postmenopausal women.

Bone health

Results from clinical studies of red clover in prevention and treatment of osteoporosis are promising but inherently complicated by interindividual variation in length of bone remodeling cycles and the bi-phasic effects of isoflavone dosings.[49] Background hormonal milieu in the body also influences outcomes,[50-53] as do basal metabolic rate,[54] active weight loss,[55] and natural aging,[56] the latter two correlating strongly with calcium absorption status in postmenopausal women.[55,56]

Five studies have examined the effects of red clover isoflavone extracts on bone mineral density or bone turnover markers (Table 3). All three of the bone mineral density (BMD) studies noted favorable effects in terms of preservation of bone. After a 1-month placebo run-in period, a 6-month study documented increased BMDs of the proximal forearm (2.9%, 4.1%, 3.0% increases, respectively), but not the distal forearm, for 25-, 50- and 75-mg daily doses of red clover isoflavones (product not stated; Table 3) in menopausal women.[8] Another 6-month study found 4.1% and 3.0% increases in BMD of the proximal radius and ulna in postmenopausal women taking 57 or 85 mg red clover isoflavones/day (Rimostil), respectively.[9] One year of treatment with daily dosing of one Promensil tablet significantly decreased the loss of lumbar spine BMD in pre- and perimenopausal women.[11] There was no effect in postmenopausal women, nor was there an effect on hip BMD for any group. It is possible, but not proven, that red clover isoflavones have preferential effects on cortical bone versus trabecular bone.

The bone marker studies all reported no changes in urinary *N*-telopeptide (putative bone resorption marker) or serum osteocalcin (putative bone formation marker) levels after treatment with red clover isoflavones, although it remains unclear how well these markers correlate with actual bone mineral density changes. The first study reported no measured changes in *N*-telopeptide and osteocalcin bone markers in perimenopausal women taking 50 mg of red clover isoflavones/day (product P-07, noncommercial formulation, Novogen, Ltd.) for 3 months.[10] The second study reported no effect of either Promensil or Rimostil on serum osteocalcin and urinary *N*-telopeptide levels after daily use for 3 months by menopausal women.[12] The yearlong Atkinson study[11] mentioned above also noted no effect of one daily Promensil tablet on bone resorption markers, despite the positive outcomes for BMD and bone mineral content, suggesting that physiological levels of bone markers are not reliable indicators of actual bone mineral density and content turnover rates.

Cardiovascular health

Vascular Effects

Arterial compliance is a measure of arterial stiffness, which correlates with the presence of atherosclerotic plaques in major blood vessels. The effect of red clover isoflavones on this parameter has been evaluated in 2 studies (Table 4). The first study[13] administered one tablet of Promensil daily for 5 weeks. The dose was then doubled to two tablets daily (80 mg isoflavones/day) for 5 more weeks. Treatment groups (both doses) showed increases in arterial compliance. The second study was a randomized, double-blind crossover trial[14] administering two tablets of each of two different products to normotensive men and postmenopausal women for 6 weeks per treatment. One product was significantly enriched in biochanin A (P-07(b); Novogen, Ltd.; noncommercial formulation) and the other was enriched in formononetin (P-083; Novogen, Ltd.; noncommercial formulation). The formononetin-enriched product produced a stronger adjusted trend toward favorable effect on systemic arterial compliance (SAC) and pulsed wave velocity (PWV), compared with the biochanin A-enriched product. Red clover isoflavone supplements may prove useful in improving arterial health and reducing risk of atherosclerosis, but more data are needed before a recommendation can be made.

Vascular endothelial function has not been firmly linked to development of atherosclerosis or hypertension, but it is hypothesized to play a role in the pathology of these diseases. Two published studies indicate that adhesion and platelet factors are not altered by red clover isoflavone supplements (Table 4). At 6 weeks in the previously mentioned Teede study,[14] plasma levels of vascular cellular adhesion molecule-1 (VCAM-1) were reduced in the group receiving 80 mg/day of the formononetin-enriched red clover extract (P-083). Administration of up to 85.5 mg/day of isoflavones (Rimostil) to postmenopausal women for 6 months did not result in altered blood levels of

coagulation factors V, VII, VIII, antithrombin III or fibrinogen.[1] Serum taken from 25 healthy postmenopausal women experiencing mild climacteric symptoms and receiving a daily dose of a soy and red clover combination isoflavone product (Phytogyn, Gynea, Barcelona, Spain; 17 mg isoflavones from soy, 38 mg isoflavones from red clover) for 6 months stimulated prostacyclin release in human umbilical vein endothelial cells.[57] Since prostacyclin can inhibit platelet adhesion and aggregation[58] in the endothelium, this may be one mechanism by which red clover isoflavones improve vascular health. Red clover isoflavones also increase endothelial nitric oxide synthase (eNOS) activity, eNOS expression, and nitrite levels in human umbilical vein endothelial cells after 48 hours of exposure.[59] This is another potential (nonimmediate, genomic) vascular mechanism of action of red clover isoflavone supplements.

Lipid Effects

Both abnormally high levels of low-density lipoprotein (LDL) and abnormally low levels of high-density lipoprotein (HDL) are linked to increased risk for atherosclerosis. Several studies have investigated the effects of red clover isoflavone preparations on serum HDL, LDL, and triglycerides (Table 5). Promensil, Rimostil and three experimental formulations, P-07, P-07(b), and P-083, have been tested for serum lipid effects. Trinovin apparently has not been evaluated for its effect on HDL, LDL, or triglycerides. These products differ from one another not only in their total isoflavone content, but also in their ratios of (daidzein + formononetin) to (genistein + biochanin A) (see Table 1). For example, Rimostil contains more formononetin and daidzein, versus genistein and biochanin A, when compared with Promensil. The levels of individual isoflavones in the Phytogyn combination product are unknown.

Promensil Lipid Effects. Seven clinical studies using Promensil have been published wherein changes in plasma lipid levels were evaluated; four reported positive results[2,12,19,21] and the remaining three reported no effect[4,13,16] on HDL, LDL or triglycerides. Of the four positive studies, two reported increased HDL in postmenopausal women consuming one[2] or two[19] tablets of Promensil for at least 1 month. Another study reported decreased triglyceride levels in perimenopausal women consuming one tablet of Promensil for 1 year.[21] In the last study,[12] triglycerides were lowered in women consuming two Promensil tablets for only 3 months, but the effect was limited to women with a baseline triglyceride level of $\geq$ 178 mg/dL.

Rimostil Lipid Effects. Four studies have examined plasma lipid effects of Rimostil. One showed negative results,[18] although subjects were type 2 diabetic patients, which is a population known to have imbalanced lipoprotein levels[60] and a high risk for heart disease.[61] One of the positive trials[15] was uncontrolled but did report increased HDL levels in postmenopausal women consuming one, two, or three tablets of Rimostil daily for 6 months. A second study[9] used the same treatment protocol and duration, but included a placebo group, and reported the same increase in HDL in postmenopausal women. Interestingly, both studies also reported reduced levels of apolipoprotein B, another specific risk factor for atherosclerosis, in the treatment groups. It may be possible that the different isoflavone balance in Rimostil, compared with Promensil, is responsible for this effect. The third study,[12] introduced above in the Promensil section, resulted in decreased triglyceride levels in women having baseline triglycerides > 178 mg/dL.

P-07 Lipid Effects. In premenopausal women, consumption of the experimental formulation P-07, containing 86 mg of red clover isoflavones/day, for 3 menstrual cycles showed no effects on total cholesterol, LDL, HDL, triglycerides, or lipoprotein(a) levels.[17] A study in pre- and perimenopausal women using the same product regimen over three months also found no effect of P-07 on plasma lipids.[10]

P-07(b) and P-083 Lipid Effects. A recent randomized, placebo-controlled, parallel cross-over, double-blind trial in men and postmenopausal women compared effects of a biochanin A-enriched red clover formulation (P-07(b)) versus a formononetin-enriched formulation (P-083).[20] The biochanin A-enriched product, but not the formononetin-enriched formulation, lowered LDL by 9.5% in men compared with baseline levels. Neither formulation affected plasma lipids in the group of postmenopausal women. This is the first report to demonstrate a gender effect of red clover isoflavones on LDL levels, and these results should be reproduced before mechanistic studies are carried out to determine the role of biochanin A in this phenomenon.

Phytogyn Lipid Effects. A soy and red clover combination product (Phytogyn, Gynea, Barcelona, Spain; 17 mg isoflavones from soy, 38 mg isoflavones from red clover)[57] produced a modest, but statistically significant, reduction in triglycerides in postmenopausal women after 6 months of therapy.

Breast effects

No studies have directly evaluated the effects of red clover isoflavone supplementation in breast cancer patients (see Table 6). In a study of high-risk women having increased breast density, 177 subjects (aged 49-65 years) with Wolfe P2/DY mammographic breast density patterns received Promensil daily for 1 year and exhibited no statistically significant changes in estradiol, FSH, or luteinizing hormone (LH) levels.[7] Importantly, no significant differences were observed between densities of breast patterns for the treatment and placebo groups. This is a positive result, because hormone replacement therapies (eg, conjugated equine estrogens plus medroxyprogesterone acetate) are known to increase mammographic density, a risk factor for breast cancer.[62] These results suggest that consumption of red clover isoflavones by women at high risk for breast cancer may be safe, although trials have not been conducted in patients with established breast cancer.

A randomized, placebo-controlled crossover pilot study administered two tablets of Promensil to 16 premenopausal and 7 postmenopausal women for one month, and reported a nonsignificant reduction in insulin-like growth factor (IGF-1) levels in the premenopausal, but not postmenopausal, women taking Promensil.[19] High serum levels of IGF-1 have been associated with an increased risk of breast cancer,[63] so these results may justify follow-up with a larger study.

A study for relief of cyclical mastalgia (breast pain) included subjects for a two-menstrual-cycle placebo run-in period, and subjects with <30% average decrease in pain compared with baseline levels (ie, low placebo response) were then randomized and administered one or two tablets daily of Promensil (40- or 80-mg of red clover isoflavones) over three menstrual cycles.[64] Breast pain was significantly reduced by 44% in the 40-mg group and 31% in the 80-mg group, compared with placebo. A 3-day increase in menstrual cycle length was recorded in the 80-mg group compared with the placebo group. This study may indicate promise for the use of red clover isoflavones by women having breast pain due to normal hormonal fluctuations. Other potential effects of red clover on the hormonal status of premenopausal women are currently unknown.

Endometrial effects

A 3-month study of 50-mg red clover isoflavones/day (P-07) in perimenopausal women found no change in the Ki-67 proliferative index of endometrial biopsies taken during the late follicular phase, nor were there changes in plasma estradiol, FSH, progesterone, or endometrial thickness.[10] Doses up to 85.5 mg of isoflavones/day of Rimostil for 6 months in postmenopausal women did not cause increased endometrial thickness or breakthrough bleeding.[9] These results suggest an inability of red clover isoflavone supplements to stimulate endometrial hyperplasia in women, at least when taken for short periods of time (Table 6).

Thyroid effects

No studies of the effects of red clover isoflavone supplements on the human thyroid have been published. One animal investigation examined thyroid function in eight ovariectomized ewes that were fed red clover silage for 2 weeks, and then after 5 months were either re-exposed to red clover or fed timothy hay (control diet).[65] Results indicated that the cross-sectional area of thyroid follicles was larger in ewes fed red clover, as was the magnitude of estrogen receptor α (immunoreactivity, compared with animals fed the control diet. Also, total and free T_3 (triiodothyronine) hormone levels increased significantly after the first and second red clover feeding experiments, while free and total T_4 (thyroxine) levels increased nonsignificantly ($p = 0.06$) after the second feeding experiment. Adams previously found that after grazing *T. subterraneum* for 60 days, thyroid gland weight increased in intact ewes.[66] There is currently a gap in the knowledge about clinical effects of red clover on thyroid functioning in humans with or without thyroid disease.

Insulin effects

Two clinical studies have investigated insulin effects of red clover isoflavones. The first study[18] dosed 16 postmenopausal type 2 insulin-independent diabetics with placebo or one tablet of Rimostil (50 mg total isoflavones) daily for 4 weeks and found no effect on glycosylated hemoglobin, an indirect measure of insulin resistance. The second trial[17] administered placebo or 86 mg total isoflavones (P-07) to 25 healthy premenopausal women for three menstrual cycles, after a one-cycle placebo run-in period. No significant changes in glucose and/or insulin concentrations were noted for the isoflavone group at the end of the study. These two studies suggest that red clover isoflavones likely do not affect glucose metabolism in pre- or postmenopausal women. However, one recent animal study found that extracts of

T alexandrinum L. (Egyptian clover) could decrease glucose and glycated hemoglobin levels and elevate insulin levels in streptozocin (streptozotocin)-induced diabetic male albino rats, whereas no effect was noted in normal animals.[67] Much work remains to elucidate whether red clover, or related *Trifolium* species, can be useful for the treatment of diabetes.

Inflammatory and antioxidant effects

There exists some in vivo support for using red clover extracts,[68] or pure isoflavones and their metabolites,[69,70] as topical treatments for prevention of ultraviolet light-induced phototoxicity and edema. This antiinflammatory ability is ascribed to the antioxidant effects of red clover extracts,[71,72] although pure isoflavones give widely varying results in different antioxidant assays.[73] The only human clinical trial to report the effect of red clover on antioxidant status dosed 16 pre- and 7 postmenopausal women with placebo or two Promensil tablets daily for 1 month. No change in vitamin C or vitamin E status was observed in the treatment groups.[19]

Chemotherapeutic and chemopreventive effects

The Hoxsey cancer formula has been surrounded by controversy ever since it was developed and marketed in the 1920s by Harry Hoxsey, whose great-grandfather reportedly invented the herbal mixture after watching a horse with a cancerous growth recover after selective grazing.[47] As described previously, red clover is a presumed ingredient of this product. It is estimated that more than half of all cancer patients use complementary and alternative medicine, and the Hoxsey therapy remains a popular herbal consumed by this population.[74-76] The American Cancer Society advises individuals with cancer not to seek treatment with Hoxsey's remedy,[77] because no peer-reviewed, evidence-based support exists for its efficacy or safety. Hoxsey's formula remains untested in controlled clinical trials, animal studies, or in vitro evaluations.

The Flor-Essence herbal formula is a mixture containing the four herbs found in the Essiac cancer remedy originally reported by Rene Caisse in 1922,[78] plus four additional plants,[74] including red clover. Some cancer patients in Canada use this preparation.[79,80] In an in vivo chemopreventive study, 120 female Sprague Dawley rats were administered either placebo or 3% or 6% Flor-Essence in water from 1 day after birth onward.[81] Animals were maintained on a phytoestrogen-free diet. All rats received one 40 mg/kg dose of dimethylbenz[a]anthracene at 50 days and were killed at 23 weeks. Although there was no significant difference in tumor emergence time between the groups, the 3% and 6% Flor-Essence treatment groups had 65.0% and 59.4% palpable mammary tumor incidences compared with 51.0% in the placebo group. Overall mammary tumor incidences at necropsy were 90.0% and 97.3% in the 3% and 6% treatment groups, respectively, and 82.5% in the placebo group. Mean mammary tumor multiplicities were 5.2 (3% Flor-Essence), 4.8 (6% Flor-Essence), and 2.8 (control). These results are opposite to the popular anecdotal belief that Flor-Essence is chemotherapeutic. Although there are no published clinical studies of this product in humans, the manufacturer has limited, collected unpublished data that are reported in a review article.[80] Flora, Inc. performed chronic toxicity tests in albino mice, rats, and dogs at various doses up to 10-fold above the recommended therapeutic dose and reported no gross organismal or organ toxicity. The manufacturer also reported good tolerance in subjects taking Flor-Essence over an undisclosed period of time for dyspeptic symptoms, and a "positive therapeutic effect in patients with secondary immune deficiency"[80] was noted. Nonetheless, the serious findings of the in vivo rat investigation are disconcerting and follow-up is required to ascertain safety of this product and of each of its eight herbal components. It is possible that the tumor-promoting activity seen in the in vivo study[81] is due to presence of compounds from the other herbal ingredients besides red clover.

SAFETY

Cancer patients

No studies have been conducted to follow the long-term survival or tumor recurrence rate of cancer patients who use red clover isoflavone supplements. Considering their mild estrogenic effects, and their ability to compete with estradiol for binding to estrogen receptors, it is hypothetically possible that isoflavones could have detrimental effects in cancer patients taking tamoxifen or other selective estrogen receptor modulator-like drugs.

Thyroid function

Thyroid disease incidence increases for menopausal women as they age[82] and isoflavones have been reported to reduce thyroid peroxidase in vivo,[83] although the clinical significance of these findings is unknown.

Inhibition of cytochrome P450 (CYP450) enzymes

Red clover isoflavones can inhibit CYP1A1, CYP1B1,[84,85] and CYP2C9[86] metabolic liver enzymes, which may cause increased plasma levels of those drugs that are metabolized via these pathways. However, no reports of clinically significant red clover–drug interactions exist in the published literature.

Adverse effects reported in clinical trials

Because the exact chemical content of commercial red clover isoflavone products is proprietary, and total isoflavone content (or a ratio of summed isoflavone content) is often reported rather than specific chemical content, it can sometimes be difficult to estimate clinical doses of individual isoflavones. This vague content labeling hinders correlation of clinical effects with specific red clover molecules. More trials involving chronic exposure of large patient populations to red clover isoflavone extracts are needed to assess long-term risks and safety.

Novogen, Ltd. provides a list of reported adverse effects, some occurring at doses as low as 40 mg of isoflavones/day, for their Promensil product. Treatment side effects occurred at incidences of $\leq 3.1\%$, and treatment groups had lower incidences of adverse effects compared with placebo (14.7%, 5%, and 15.6% for 40, 80, and 120 mg of isoflavones, respectively, versus 24.7% for placebo). These effects include: breast tenderness, swollen neck glands, increased thyroid function, migraine/headache, dizziness, vertigo, tremor, hypertension, acne, rash, pruritus, psoriasis, bloating, constipation, diarrhea, nausea, mouth ulcer, sore throat, myalgia, osteoarthritis, bronchitis, low platelets, reflux (80 mg), epistaxis (80 mg), menstrual bleeding (80 mg), urinary tract infection (120 mg), and vaginal thrush (80 mg). Compounds and mechanisms responsible for triggering adverse events are currently unknown.

Other safety considerations

Red clover is considered a class 2b herb by the American Herbal Products Association and as such is contraindicated during pregnancy.[87]

CONCLUSIONS

Red clover botanical dietary supplements presently are being used for treatment of "off-label" conditions, at least in the historical context of this plant's traditional uses. Modern uses are mostly premised on the estrogenic activity of red clover, which is attributed to the four isoflavones daidzein, genistein, formononetin and biochanin A. Studies of red clover supplements for relief of menopausal hot flashes have been generally disappointing, although their interpretation is unclear because of design flaws such as short duration, low dose, and nonuniform entrance qualifications for women, such as variable LH level and BMI cutoffs. Studies using red clover supplements to preserve bone health show more initial promise, but follow-up studies are needed to determine the specific types and locations of bone(s) that receive benefit from isoflavones. Also, bone endpoint selection is critical; studies measuring bone marker levels versus actual bone mineral density or bone mineral content may yield different results. Red clover isoflavone supplements do seem to significantly improve arterial elasticity, which one would expect to decrease atherosclerosis risk, and these findings require confirmation and elaboration. Enriched red clover isoflavone supplements do not have consistent, clear effects on LDL or HDL levels in most menopausal women, but biochanin A-rich supplements may selectively lower LDL in men. Red clover isoflavones do not seem to stimulate the endometrium or breast in the few investigations conducted thus far.

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