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Review

Effectiveness of Commercial Red Clover (*Trifolium pratense* L.) Products for the Treatment of Symptoms in Menopausal Women—A Narrative Review

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Abstract: Red clover (*Trifolium pratense* L.) is found in southeast Europe and Anatolia. Its primary traditional medicinal use includes the treatment of various conditions of the upper respiratory tract. In recent years, its isoflavones have become the focus of research aimed at developing treatments to alleviate menopausal symptoms. Reduced levels of circulating estrogen due to reduced ovarian function can cause short-term symptoms such as hot flashes, palpitations, difficulty sleeping, headaches, fatigue, mood disorders and reduced concentration but also long-term chronic conditions, such as cardiovascular disease, accelerated weight and bone mass loss, atrophic vaginitis, osteoporosis, and cognitive impairment. The aim of this narrative review was to analyze the effects of commercially available and standardized red clover extracts on menopausal women. Eight randomized controlled trials on a total of 8769 menopausal women (aged 40 to 65 years) evaluated the effect of red clover isoflavone extract on menopausal symptoms. In all studies, isoflavone extract treatment showed improvement in all menopausal symptoms, including some common comorbidities, namely, hot flashes (1487 women, 25%), blood lipids (1155 women, 19%), atherosclerosis (6938 women, 79%), risk of breast cancer and endometrial cancer (428 women, 5%), osteoporosis and osteopenia (555 women, 6%), and menopause-related cognitive impairment (3530 women, 40%).

Keywords: red clover; *Trifolium pratense* L.; menopause; red clover extracts; bioactive compounds

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1. Introduction

Menopause is the physiological or iatrogenically induced cessation of menstruation (amenorrhea) due to reduced ovarian function. The decrease in circulating estrogen levels can cause menopausal symptoms, including short-term symptoms such as hot flashes, palpitations, sleep difficulties, headaches, fatigue, mood disorders, and decreased concentration, as well as long-term chronic conditions such as cardiovascular diseases, accelerated bone loss, atrophic vaginitis, osteoporosis, and cognitive impairment [1]. Perimenopause refers to the years before (duration varies widely) and one year after the last menstruation. Perimenopause is usually characterized by an increased frequency of menstruation followed by thinning (*oligomenorrhea*), but any pattern is possible; conception is still possible during perimenopause. The period after menopause is called postmenopause [2]. The term "climacteric" comes from the Greek word "klimakter", meaning change, and encompasses the entire period of gradual ovarian function decline from perimenopause, through menopause, including postmenopause [3]. Premature menopause, also known as premature ovarian failure, is defined as the cessation of menstruation before the age of 40 and can be caused by factors such as smoking, living at high altitudes, and malnutrition [4]. Iatrogenic (artificially induced) menopause can occur due to medical procedures such as oophorectomy, chemotherapy, pelvic radiation, or any other procedure that disrupts blood supply [5].

As the ovaries age, their response to pituitary gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) weakens, initially causing shortened follicular phases (with shorter and irregular cycles), less frequent ovulations, and consequently reduced progesterone production [6]. Eventually, the follicles become unresponsive, resulting in limited estradiol production. Estrogens (now mainly estrone) still circulate; they are produced by peripheral tissues such as adipose tissue and skin from androgens like androstenedione and testosterone [7]. However, the overall estrogen level is much lower. Around menopause, androstenedione levels decrease by half, but the decline in testosterone, which begins gradually during young adulthood, does not accelerate during menopause as the stroma of the postmenopausal ovary and the adrenal glands continue to secrete significant amounts [8].

The diagnosis of physiological menopause is clinically confirmed when menstruation is absent for one year. The aforementioned manifestations or symptoms can be treated (e.g., with hormones or selective serotonin reuptake inhibitors (SSRIs, antidepressants)). In the United States, the average age of physiological menopause is 51 years, while in Croatia, the average age of menopause is 48.8 years [9,10].

The aim of this narrative review was to analyze the effects of commercially available standardized extracts of red clover (*T. pratense* L.) on women's health during menopause. Eight randomized controlled trials were included in this review; all assessed the effect of red clover isoflavone extract on menopausal symptoms [1,8,9,11–16].

2. Botanical Origins

Clover (*Trifolium*) is one of the most important legumes (*Fabaceae*) in temperate and humid regions. It plays a crucial role in livestock nutrition [8] and makes a significant contribution to agricultural and animal production in Europe and America. Red clover (*Trifolium pratense* L.) naturally grows in southeastern Europe and Anatolia, with Anatolia being recognized as its homeland [17].

The Latin name of the genus *Trifolium* comes from the Greek word *trifolion* (three-leaved), a compound of the words *tria* (three) and *folium* (leaf). The species name *pratensis* (meadow) indicates the plant's habitat. Red clover is a herbaceous perennial plant belonging to the legume family (*Fabaceae*). It naturally grows in the regions of Europe and western Asia and is often found as a real green carpet in meadows and pastures. It reproduces through seeds. In agriculture, red clover is considered a significant plant. Clusters of symbiotic bacteria form on its roots, which bind nitrogen from the air and thereby fertilize the soil. In addition to enriching the soil by giving it fertility, it is also important as a fodder plant. It is an excellent source of nectar and can contribute to substantial honey production. Daily intake can reach up to 3 kg per hive, yielding a total of up to 260 kg of honey per hectare [18]. Seven diploid varieties (2n = 2x = 14) have been identified worldwide. It serves as a natural source of valuable isoflavonoids, utilized in commercial products (e.g., Menoflavon, Rimostil, Promensil) in some parts of the world [17].

The stem is upright, highly branched, and sparsely hairy, growing up to 50 cm. The root system is a taproot, well-branched, ranging from 50 to 100 cm in length, with round nodules. The leaves are alternate, elliptical, 2–4 cm long, and 1–1.5 cm wide, with a distinct white triangular spot, arranged in groups of three on a hairy stalk. At the base of the stalk, there are two membranous stipules. The lower leaves are on stalks around 20 cm long, while the upper ones are short-stalked or nearly sessile [19]. The flowers are hermaphroditic, irregular, clustered in head-like inflorescences on a 1–7 cm long, hairy stalk. The inflorescence is compound. The calyx is bell-shaped, covered with hairs, and has five triangular lobes. The corolla resembles a butterfly, red in color, and is twice as long as the calyx. The ovary has an elevated stigma carrying 1–2 embryonic seeds, with 10 stamens, 9 of which are fused into a tube. Blooming occurs from May to autumn [20]. The fruits are rounded pods containing 1–2 elongated-ovate, smooth seeds [18].

It can be sown alone for hay production or mixed with cereals in crop rotations. It can be cultivated in various soil types, pH values, and environmental conditions, providing

good yields [21]. Tetraploid *Trifolium pratense* L., obtained through classical hybridization methods, is an important forage plant in Asia, Europe, North America, New Zealand, and Australia. Records show that in the year 2000, clover seed was produced on 25,422 hectares in European Union member states [red clover (*T. pratense* L.) on 11,031 ha; white clover (*T. repens* L.) on 4346 ha; and other clover species on 10,045 hectares]. France had the largest area for red clover production (5732 hectares), followed by Sweden [22].

3. Bioactive Compounds from Red Clover with Beneficial Health Effects

Red clover contains a number of potent bioactive compounds including polyphenols, flavonoids, coumarin derivatives, cyanogenic glycosides, and volatile oils, as well as vitamins and trace minerals [23], which have been identified in parts of the plant in various quantities, as summarized in Table 1.

Table 1. Bioactive compounds and their quantities identified in all parts of *Trifolium pratense* L.

Plant Part	Bioactive Compounds	Quantity (mg/g in Dry Metter dm)	Reference
Roots	formononetin	2.88	[8,23–26]
	biochanin A	1.95	
	maackiain	2.68	
	pseudobaptigenin	2.19	
Leaves	biochanin A	39.3	[25–30]
	formononetin	32.2	
	genistein	23.4	
	daidzein	5.1	
	glycitein, irilone, quercetin, ononin, maackain, orobol, pratensein, pseudoapigenin, prunetin, prunetin-4-glucopyranoside, genistein-7-galactopyranoside, pinitol-6-methoxycyclo-hexanepentaol	<1	
Stems	formononetin	34.9	[18,28,30]
	biochanin A	18.7	
	genistein	2.37	
	daidzein	34.9	
	prunetin, glycitein, pseudoapigenin, pratensein, irilone, orobol, prunetin-4-glucopyranoside, genistein-7-galactopyranoside, pinitol-6-methoxycyclo-hexanepentaol	<1	

Table 1. *Cont.*

Plant Part	Bioactive Compounds	Quantity (mg/g in Dry Metter dm)	Reference
Seeds	biochanin A	0.93	[24,25,28–31]
	formononetin	0.83	
	genistein	0.64	
	daidzein	0.20	
	flavonol quercetin, taxifolin dihydroxyquercetin, soyasaponin I, 22-O-glycosides, 22-O-diglycosides, astragaloside VIII, prunetin, prunetin-4-glucopyranoside, pinitol-6-methoxycyclohexanepentaol	<1	
Flowers	biochanin A	37.4	[28,30]
	formononetin	23.6	
	genistein	34.1	
	daidzein	4.9	
	prunetin, genistein, prunetin-4-glucopyranoside, genistein-7-galactopyranoside, pinitol-6-methoxycyclohexanepentaol	<1	

In phytotherapy, the flower heads of red clover are used for tinctures, which are collected during the flowering season. Red clover flower juice is used for eye diseases [18]. The red clover concentrate (obtained by extraction by continuous boiling in water) and infusion (soaked in cold or hot water without boiling) are still used as expectorants (means of facilitating the expectoration of tracheal secretions), alternatives (improving the state of body fluids), sedatives, and rheumatism drugs. Red clover is occasionally used to induce menstruation or as a fertility tonic [32–34].

Today, the well-known and clinically tested commercial products, available in North America that contain red clover are Promensil, Rimostil, and Trinovin, all produced by Novogen, Ltd. (North Ryde, NSW, Australia). Given the extent of the available clinical evidence for the aforementioned commercial products, the majority of the evidence presented in this review is based on these particular products.

4. Use of Red Clover in the Treatment of Perimenopause and Postmenopause Symptoms

In the 19th and 20th centuries, infusions (flower tea) or tinctures (ethanol extract) were used to treat upper respiratory tract diseases such as cough, asthma, bronchitis, laryngitis, and tuberculosis. They were also employed as mild sedatives, antispasmodics (for whooping cough, measles), and remedies for rheumatism. Creams and ointments were used for the treatment of burns, wounds, gout, and fungal infections. Southeastern Cherokee Indians used flower tea or aerial parts to treat fever, Bright's disease (chronic inflammation of the kidneys, nephritis), leukorrhea (vaginal discharge), and as a "blood medicine". The Southern Ute Indian tribe, native to Colorado, used red clover syrup as an abortifacient, and they rolled red clover leaves into cigarettes, using them to treat asthma [35–38].

Commercial products containing red clover were introduced to the market in the early 20th century for the treatment of various health conditions. In the year 1900, the Wm. S. Merrell Chemical Company (Cincinnati, OH, USA) produced a trifolium extract for treating syphilis, scrofula, chronic rheumatism, and various skin issues. In the year 1920, Harry Hoxsey and Norman Baker introduced Hoxsey as a cancer remedy. For

the same application, in 1934, Flora, Inc. (Lynden, WA, USA) and Flora Manufacturing & Distributing, Ltd. (Burnaby, BC, Canada) produced Flor-Essence or "Indian tea".

The growing interest in red clover isoflavones can be attributed to research on the estrogenic effects of *Trifolium* species on grazing and experimental livestock. Given that red clover isoflavones show structural similarities to endogenous 17β-estradiol, they can exert their biological effects via activating the estrogen receptor (ER), showing higher affinity for ER-β than for ER-α. In addition, numerous nonhormonal effects of isoflavones have been reported, including tyrosine kinase inhibition, antioxidant activity, and effects on ion transport [39–41].

Over the past few years, semipurified isoflavone supplements from red clover have been studied for the use in menopause, improving bone health and maintaining the cardiovascular system, and in the treatment of breast, ovarian, and prostate cancer. A summary of the bioactive compounds that have shown effects on the symptoms and common comorbidities of menopause is shown in Table 2.

Table 2. Bioactive compounds isolated from red clover (*Trifolium pratense* L.) plant parts with positive effects on the common symptoms and/or comorbidities during menopause.

Menopausal Symptoms and/or Comorbidities	Aglycones of Red Clover Isoflavone Extract	Quantity (mg/g dw)	Red Clover Plant Parts	References
Hot flashes	biochanin A	7.29 ± 1.29	leaves	[1,8,11–16,28,42,43]
		2.81 ± 0.04	stems	
	formononetin	8.15 ± 3.14	leaves	
		8.87 ± 2.61	stems	
	genistein	0.62 ± 0.23	leaves, stems	
		0.27 ± 0.17	stems	
		0.46 ± 0.12	aboveground parts	
	daidzein	0.55 ± 0.35	leaves	
		0.07 ± 0.06	stems	
Blood lipoprotein composition	formononetin	8.15 ± 3.14	leaves	[1,8,11–16,26,28,44]
		6.38 ± 1.95	stems	
	biochanin A	8.12 ± 1.21	leaves	
		2.80 ± 0.65	stems	
	genistein	0.69 ± 0.03	leaves	
		0.31 ± 0.22	stems	
		0.34 ± 0.11	aboveground parts	
	daidzein	0.55 ± 0.35	leaves	
		0.07 ± 0.06	stems	
Atherosclerosis	biochanin A	9.05 ± 2.69	leaves	[1,8,11–16,28,45]
		2.66 ± 0.79	stems	
	genistein	0.59 ± 0.30	leaves	
		0.13 ± 0.05	stems	
		0.22 ± 0.04	aboveground parts	
	biochanin A	7.29 ± 1.29	leaves	
		3.08 ± 0.96	stems	

Table 2. *Cont.*

Menopausal Symptoms and/or Comorbidities	Aglycones of Red Clover Isoflavone Extract	Quantity (mg/g dw)	Red Clover Plant Parts	References
Anticancer effects	biochanin A	6.46 ± 1.61	leaves	[1,8,11–16,28,45]
		2.33 ± 1.74	stems	
	formononetin	8.15 ± 3.14	leaves	
		10.32 ± 3.63	stems	
Bone health	formononetin	8.15 ± 3.14	leaves	[1,8,11–16,26,28,46]
		17.51 ± 2.24	stems	
	biochanin A	5.77 ± 0.81	leaves	
		1.39 ± 0.34	stems	
Cognitive effects	formononetin	8.15 ± 3.14	leaves	[1,8,11–26,28]
		10.53 ± 3.50	stems	
	biochanin A	8.30 ± 2.15	leaves	
		2.30 ± 1.36	stems	

In plant isoflavones, glycosides are prominent. The hydrolytic conversion of glycosides to aglycone analogs is required to facilitate absorption. Fermented isoflavone aglycone preparations show increased bioavailability compared to that of similar glycosides. The use of enzymatic techniques and probiotics has been proven to enhance the uptake of these compounds, thereby improving the efficacy of isoflavone treatment [47].

Despite the well-known benefits of hormone therapy in menopause, due to the potential serious side effects and the risk of breast cancer, the use of this alternative therapy, even in the treatment of hot flashes, remains controversial [45,48]. Many women have discontinued hormone therapy after the publication of the Women's Health Initiative study results, seeking an effective and safe alternative to alleviate menopausal symptoms [49,50].

The reluctance to accept hormone therapy, which has been associated with concerns about its safety, has led to the popularization of many alternative and complementary treatment methods [42,51,52]. For several years, red clover has been one such alternative that women use to treat menopausal symptoms, including hot flashes [40].

4.1. Hot Flashes

The most common symptom of menopause are hot flashes, which can last several years after menopause. About 70% of women report experiencing them, with differences in different populations [53,54]. The frequency of hot flashes depends on climate conditions, race/ethnicity, but also diet and lifestyle, as well as women's personal attitude toward the end of reproductive life and aging [55–57]. Sometimes, the intensity of hot flashes can be so significant that treatment should be considered, since women report sleep and mood disturbances that eventually negatively impact their daily activities at home or at work, and the overall quality of life [58,59]. Hot flashes are thought to be the result of the brain's response to progressive estrogen deficiency and fluctuations in neurotransmitter activity, especially in the serotonergic and noradrenergic pathways, leading to instability of the hypothalamus thermoregulation mechanism. Ultimately, this results in increased blood flow to the skin and the enhanced activity of sweat glands, causing these symptoms [54,60].

Lambert et al. [11] launched one of the first studies addressing the impact of combined red clover isoflavones and probiotics on the vasomotor symptoms of menopause. They included the objective skin conductance (SC) capture of hot flashes and the determination and standardization of the concentration and molecular form of the isoflavone component of red clover extract (RCE); with a combined methodology, they considered participant

characterization and eligibility [61]. They conducted a parallel, double-blind, randomized controlled trial of 62 women from the northern Denmark region in perimenopause, aged 40–65 years, with a BMI of 20–40, who reported ≥ 5 hot flashes/day and had follicle-stimulating hormone (FSH) levels ≥ 35 IU/L.

Each participant, twice a day for 12 weeks, received treatment with bioavailable RCE, providing 34 mg/d of isoflavones and probiotics, or a masked placebo formulation. The bioavailable RCE was obtained by adding a heterogeneous culture of lactic acid probiotic bacteria to the red clover extract to facilitate cold fermentation and improve bioavailability [62]. The standardization of the aglycone content after fermentation was confirmed by liquid chromatography–mass spectrometry (LC-MS) by DB Lab A/S, (Odense, Denmark). With the purpose of improving the characteristic taste and appearance of RCE, stevia and sugar-free berry/orange aroma were added.

The primary outcome was the change in the daily frequency of hot flashes from baseline to 12 weeks using 24 h SC (ambulatory skin conductance). Secondary outcomes included changes in SC-determined intensity (HFI), self-reported HFF (rHFF), severity of hot flashes (rHFS), blood pressure, and plasma lipids [63]. A significant reduction in 24 h HFF was observed when comparing the change from baseline to 12 weeks of RCE treatment with that of the placebo. rHFF was also significantly reduced in the RCE group compared to that in the placebo group. Other parameters were not significantly different. RCE was well tolerated. The results suggested that moderate doses of RCE were more effective and superior to the placebo in reducing physiological and self-reported vasomotor symptoms (hot flashes, flushing, and night sweats).

Kanadys et al. [1] conducted a meta-analysis on the effectiveness of red clover isoflavones in alleviating hot flashes and symptoms in perimenopausal and postmenopausal women. Twelve randomized, placebo-controlled clinical trials were selected for the analysis, with trial durations ranging from 12 weeks to 2 years. The clinical studies were conducted in Australia, Peru, the Netherlands, the United States, the United Kingdom, Ecuador, Brazil, Austria, Iran, and Denmark. In total, 1179 menopausal women participated in the studies, with sample sizes ranging from 37 to 252 (1043 participants included in the final analysis). Eight trials included postmenopausal women, three studies included women in peri- and postmenopausal periods [64–66], and women in perimenopause were included in one study [11]. The average dose of red clover isoflavones was 65.1 mg/d of aglycone equivalent (range 37.1–160 mg/d). Two studies included two therapeutic groups with different doses of isoflavones [64,67]. The compositions of the isoflavones and their doses varied among the studies. Eight studies measured the daily frequency of hot flashes (≥3/day). Ten studies evaluated the presence and/or severity of various somatic and psychological symptoms using menopause symptom assessment scales. In most studies, a dose of 40–80 mg/d of RCIE was used, except in [5], where 37.1 mg/d of aglycone was applied. Out of eight RCTs with ten comparisons assessing the frequency of hot flashes, six [11,42,64,67,68] demonstrated a reduction in hot flashes, including four significant reductions [11,42,68] in the isoflavone group compared to a placebo group; in four comparisons, no differences were observed between the groups [64,66,67,69].

The meta-analysis of eight studies (ten comparisons) demonstrated a statistically significant reduction in the daily incidence of hot flashes in women receiving red clover compared to that in those receiving a placebo [11,42,65–67,70,71]. Due to 87.34% homogeneity, the analysis showed a significant difference in postmenopausal women with ≥5 hot flashes per day when the follow-up period was 12 weeks, with an isoflavone dose of ≥80 mg/day, and when formulations contained a higher proportion of biochanin A.

Booth [14] conducted an analysis of studies administering semipurified red clover preparations to women for alleviating menopausal hot flashes. The examined studies provided short-term results (≤12 weeks), and the achieved positive effects of the preparations required 8 weeks to manifest. Six short-term trials (≤4 months) investigated the effectiveness of red clover isoflavone supplements in reducing hot flashes, of which only three studies, all using Promensil, yielded positive results. A two-month noncontrolled study

provided 23 menopausal women (aged 40–65 years) one Promensil tablet per day, reaching a 56% reduction in the frequency and a 43% reduction in the intensity of hot flashes. In the second double-blind, randomized, placebo-controlled study, 51 postmenopausal women (aged 45–65 years) were randomized to receive one Promensil tablet per day or placebo over 3 months. The two groups did not differ significantly in the frequency of hot flashes. Among one or four Promensil tablets per day over the 3 months, 37 postmenopausal women (aged 40–65 years) also did not differ in terms of the frequency of hot flashes. The authors, however, noted a significant placebo response.

A 4-month, randomized, double-blind study evaluated the effect of one tablet of Promensil per day in comparison to that of a placebo on the frequency and intensity of hot flashes in 30 Peruvian postmenopausal women (younger than 60 years; median age 52 ± 0.7 years) [70]. The frequency of hot flashes decreased by 49% and 11% in the treated and placebo groups, respectively, and the intensity of hot flashes decreased by 47% compared to 0% in the placebo group. Another double-blind, randomized, placebo-controlled study encompassing 30 Dutch postmenopausal women (aged 49–65 years) showed that two Promensil tablets per day in comparison reduced the frequency of hot flashes by 44% compared to placebo after 12 weeks [68]. The authors also reported a small change in the mean body mass index (BMI) in the treated group in comparison to the placebo group [70].

Tice et al. [64] conducted a double-blind, randomized, placebo-controlled study on 252 women aged 45 to 60 years, finding a similar reduction in hot flashes after 12 weeks in the two treatment arms (Promensil, 82 mg isoflavones/day, high genistein + biochanin A; Rimostil, 57 mg isoflavones/day, high daidzein + formononetin) in comparison to a placebo. Although the overall results did not reach statistical significance, a slightly better response was found in women with BMI > 25 kg/m^2 regardless of active treatment arm, suggesting metabolic effect of red clover isoflavones.

Shakeri et al. [15] conducted a randomized, triple-blind, placebo-controlled clinical trial involving 72 healthy postmenopausal women over 12 weeks. The intervention arm received two capsules containing 40 mg of dried red clover leaves per day. The placebo was two capsules containing 40 mg of starch per day. The outcome measures were menopausal symptoms determined using the Menopause Rating Scale (MRS) [71]. The overall MRS score decreased from 20.41 to 10.08 in the intervention group and from 20.77 to 17.20 in the control group [72], which was attributed to scores in the vegetative-somatic and psychological categories of menopausal symptoms. In comparison to the placebo, dried red clover leaves were more effective in reducing the severity of vasomotor and menopausal symptoms, including mood disorders, especially symptoms of anxiety and depression.

Akbaribazm et al. [8] conducted a randomized, double-blind, placebo-controlled study. They reviewed 80 related articles on the beneficial effects of red clover on biological processes involving the participation of 190 postmenopausal women. The research results showed that the ethanolic extract of the aerial parts of red clover (398 mg/day standardized to 120 mg of isoflavones) significantly reduced hot flashes and vasomotor symptoms in the studied women after 12 months [64].

4.2. Blood Lipoproteins

The potential effect of red clover isoflavones on lipid metabolism has been proven in animal models [73,74], while human findings have been mixed. Some studies showed a blood-lipid-lowering effect [75–77], while others found no change in lipid status [44,78].

Kanadys et al. [13] conducted a systematic review and meta-analysis with the aim of explaining the effect of a specific standardized isoflavone extract of red clover on the lipid profile of peri- and postmenopausal women. Ten studies, with interventions between 12 weeks and 12 months, fit the inclusion criteria, encompassing a total of 910 menopausal women, average age 53.9 (±4.1), (range 40–85). The studies were conducted in the United Kingdom, Ecuador, Australia, Denmark, Serbia, and the United States [79]. The mean dose of red clover isoflavone (RCI) extract was 61.5 mg per day as aglycone equivalents (range,

33.8–160 mg per day). The meta-analysis confirmed that red clover extract is effective in reducing the concentration of total cholesterol but not the levels of HDL-C, LDL-C, and triglycerides [13].

Booth [14] reviewed several studies investigating the effects of red clover isoflavone preparations on arthrosis risk, namely serum concentrations of HDL, LDL, and triglycerides. Studies included in the review tested the effects of Promensil, Rimostil, and three experimental formulations, P-07, P-07(b), and P-083, on serum lipids. All observed products differed significantly in both the total contents of isoflavones and their ratios (daidzein + formononetin vs genistein + biochanin A). These differences prevented the proper comparison since, for example, Rimostil, in comparison to Promensil, contains more formononetin and daidzein relative to genistein and biochanin A.

Four out of seven studies that assessed the effects of Promensil on plasma lipid levels had positive results [65,67,78,79], and the remaining found no effect [44,80,81] on HDL, LDL, or triglycerides. Two of the four studies with positive results reported an increase in HDL in postmenopausal women who consumed one or two tablets per day of Promensil for at least one month [67,78]. Reduced triglycerides in perimenopausal women consuming one tablet of Promensil during 12 months was reported in another study [65]. In a more recent study, lower triglycerides were found in women who consumed two Promensil tablets for only three months, but the effect was limited to women with baseline triglyceride levels > 178 mg/dL [80].

Four clinical studies examined the effect of Rimostil on plasma lipids [69,76,80,82]. One study, with negative findings [83], was conducted on patients with type 2 diabetes, a population known to have imbalanced lipid levels and a high risk of heart disease [84,85]. Positive findings, specifically increased HDL levels, were reported from an uncontrolled study in postmenopausal women who consumed one, two, or three Rimostil tablets daily for six months [69]. Another study, which used the same treatment protocol and duration as that previously mentioned but included a placebo, reported an increase in in HDL levels in postmenopausal women [76]. Interestingly, both studies [69,76] also reported reduced levels of apolipoprotein B in the treatment groups, another specific risk factor for atherosclerosis. Possibly, the reason lies in the different balances of isoflavones in Rimostil as compared to Promensil. Reduced triglyceride levels in women with baseline values > 178 mg/dL after using Promensil were found, as previously mentioned [79].

In perimenopausal women, experimental formulation P-07, containing 86 mg of red clover isoflavones per day consumed over 3 menstrual cycles, showed no impact on the total cholesterol, LDL, HDL, triglycerides, or lipoproteins [77]. The use of P-07 for 3 months in women before and during perimenopause had no effect on plasma lipids [86].

One study reported gender-specific effect of red clover isoflavones on LDL levels. This randomized, placebo-controlled, crossover, double-blind study encompassed men and postmenopausal women. The study used a red clover formulation enriched with biochanin A [P-07(b)] and a formulation enriched with formononetin (P-083) [44]. Only the P-07(b) product showed a 9.5% reduction in LDL in men only. Neither formulation affected the plasma lipid levels in postmenopausal women.

Clinical studies assessing the effects of the use of Promensil and Rimostil on serum lipids were reviewed by Akbaribazm et al. [8]. In a double-blind, randomized, placebo-controlled trial, supplementation with a 50 mg Rimostil tablet for 2 years reduced triglyceride and LDL levels and increased HDL levels in 189 menopausal women [87]. In another randomized, double-blind, placebo-controlled prospective study on 37 postmenopausal women with symptoms of estrogen deficiency, treatment with 40 mg/kg of Promensil in comparison to a placebo for 12 weeks had the same results: significantly lower triglyceride and LDL levels and increased HDL levels [67].

The administration of 40 mg of biochanin A to 19 women with premenstrual syndrome over 12 weeks in a randomized, double-blind, placebo-controlled trial had reduced triglyceride and LDL levels and increased HDL levels and reported significant reductions in premenstrual syndrome symptoms such as fatigue and swelling [88].

4.3. Atherosclerosis

Atherosclerosis is a general term for several conditions in which the artery wall becomes thinner and less elastic. The most common and important form is atherosclerosis, where the accumulation of fatty material occurs beneath the inner sheath (endothelium) of the arterial wall [5]. As a late symptom of menopause, as a consequence of a change in fat metabolism, lipoproteinemia alters the intensity of the atherosclerosis process. Consequently, high blood pressure is a common presentation. These factors contribute to increased risks of heart attack and stroke during menopause [89].

Arterial compliance, a measure of arterial stiffness, correlates with the presence of atherosclerotic plaques in major blood vessels. The first study [44] administered one Promensil tablet daily for 5 weeks. The dose was then doubled to two tablets per day (80 mg isoflavones/day) for an additional 5 weeks. Both treatment groups (both doses) showed an increase in arterial compliance. The second study was a randomized, double-blind crossover study that gave two tablets of two different products to normotensive men and postmenopausal women for 6 weeks per treatment. One product was significantly enriched with biochanin A [P-07(b); Novogen, Ltd.; noncommercial formulation], and the other was enriched with formononetin (P-083; Novogen, Ltd.; noncommercial formulation). Product P-083, enriched with formononetin, had a more favorable effect on arterial compliance than product P-07(b), which was enriched with biochanin A.

Vascular endothelial function has not been firmly linked to the development of atherosclerosis or hypertension but is assumed to play major role in its pathology. Based on the findings of two studies, supplementation with red clover isoflavones showed no effect on platelet adhesion or aggregation factors. After 6 weeks in the study by Teede et al. [90], the plasma levels of vascular cell adhesion molecule-1 (VCAM-1) were reduced in the group receiving 80 mg/day of red clover extract enriched with formononetin (P-083). The administration of up to 85.5 mg/day of isoflavones (Rimostil) to postmenopausal women for 6 months did not result in changes in coagulation factors V, VII, VIII, antithrombin III, or fibrinogen in the blood [69]. The serum taken from 25 healthy postmenopausal women with mild menopausal symptoms, receiving a daily dose of a combination of soy and red clover isoflavone product (Phytogyn, Gynea, Barcelona, Spain; 17 mg soy isoflavones, 38 mg red clover isoflavones) for 6 months, showed that the treatment stimulated the release of prostacyclin in the endothelial cells of human umbilical veins [91]. Since prostacyclin can inhibit platelet adhesion and aggregation in the endothelium, this could be one of the mechanisms by which red clover isoflavones improve vascular health. Red clover isoflavones also increased the activity of endothelial nitric oxide synthase (eNOS), eNOS expression, and nitrite levels in the endothelial cells of human umbilical veins after 48 h of exposure [92]. This is another potential (direct, genomic) vascular mechanism of action of red clover isoflavone supplements.

Akbaribazm et al. [8] conducted a systematic review of clinical studies to explain the effects of a specific standardized extract of red clover isoflavones on the cardiovascular system of postmenopausal women. Flavonoids have various effects, including anti-inflammatory, antiangiogenic, and antiallergic effects. The transcription factor NF-kB plays a role in inducing the expressions of inflammatory mediators such as cytokines, cell surface receptors, adhesion molecules, and acute-phase proteins. Treatment with genistein (0.3 mg/kg), a component of red clover, for 8 weeks inhibited the development of atherosclerosis by inhibiting the expressions of NF-kB, thrombin, TNF-α cytokines, and vascular cell adhesion molecule-1 (VCAM-1) in LDL receptor knockout mice [93]. These factors (i.e., NF-KB, TNF-a, and VCAM-1) contribute to the development of atherosclerosis by inducing monocyte accumulation and promoting monocyte adhesion to the vessel wall at sites prone to atherosclerotic lesion formation [94]. Furthermore, the Fas/Fas ligand system has been identified to be under the control of the estrogen receptor in monocytes. This suggests a link between estrogen and many disorders, including atherosclerosis, vascular inflammation (vasculitis), and rheumatoid arthritis [95].

The risk of cardiovascular diseases increases after menopause. In a 20-year follow-up of 2873 women younger than 55 years within the Framingham study, the annual incidence of cardiovascular diseases (coronary heart disease, stroke, and congestive heart failure) was reported as 0.6–5% per 1000 women in perimenopausal women aged <40 and 50–54 years [96]. A randomized, double-blind, placebo-controlled trial [97] found that the administration of drospirenone (2 mg) plus β-estradiol (1 mg) over a 13-month period significantly lowered blood pressure and endometrial bleeding in 1142 postmenopausal women. The most likely explanation for these observations is the protective effect of endogenous female sex steroids, especially estrogen, during the years before menopause [98]. In various studies, the effects of raw extracts and compounds isolated from red clover on heart and vascular diseases have been investigated. The use of commercial products containing the active ingredients from red clover, including biochanin A and formononetin, increased vascular vasoconstriction, SAC, and the speed of the pulmonary artery. The reason for these effects may be associated with the release of vasoconstrictor compounds, including NO, prostaglandins (PGs), and endothelial hyperpolarization factor. Calcium is essential for the contraction of the smooth muscle cells in the vascular wall; however, formononetin inhibits the intracellular influx of Ca into vascular smooth muscle cells [48].

4.4. Breast and Endometrium Carcinoma

Breast cancer stands as the leading cause of death among women from all cancerous diseases [99]. The introduction of the antiestrogen drug tamoxifen has significantly improved the health and overall survival of women with estrogen/progesterone diseases [100,101]. However, in perimenopausal women, antiestrogens induce early menopausal syndrome, which are often poorly tolerated [102], leading to therapy discontinuation in a considerably high percentage of cases [103,104].

Ferraris et al. [12] conducted a systematic review of clinical studies with the aim of determining whether a red clover preparation together with a dietary intervention applied in perimenopausal women with breast cancer (BC) improves menopausal symptoms due to antiestrogen treatment and therefore promotes tamoxifen compliance, prevents weight gain, and is safe.

Surgically treated perimenopausal women with estrogen receptor-positive (ER+) diseases taking tamoxifen were engaged in a prospective, double-blind, randomized trial [99]. The red clover group (n = 42) received one oral tablet daily of an 80 mg pharmaceutical extract of red clover for 24 months. The placebo group (n = 39) received one oral tablet daily without an active ingredient. All women were encouraged to adopt a Mediterranean-type diet and to remain active. Outcomes included menopause assessment (MRS), body mass index (BMI), waist and hip circumference, insulin resistance, and levels of cholesterol, triglycerides, and sex hormones. The safety indicators investigated were endometrial thickness, breast density, and the effects of patient serum on ER-positive breast cancer cell lines [105].

The red clover group exhibited significantly greater reductions in BMI and waist circumference. HDL cholesterol significantly increased in both groups. The levels of total cholesterol, LDL cholesterol, triglycerides, insulin resistance, and sex hormones did not significantly vary during the study period and did not differ between groups. Snack and red/processed meat consumption significantly decreased in both groups, while the consumption of unrefined grains, legumes, fish, nuts, and seed oils significantly increased. Physical activity also significantly increased. Endometrial thickness remained constant. Breast density significantly decreased in both groups. Proliferation and estrogen-regulated gene expression did not differ in the cell lines treated with the serum from each group.

The first study assessing red clover in tamoxifen-treated patients with BC, containing isoflavones, proved the treatment was clinically and in vitro safe and was associated with reduced BMI and waist circumference. However, dietary and lifestyle interventions likely improved menopausal symptoms.

Booth [14] analyzed five clinical studies, none designed to specifically assess the effect of red clover isoflavone supplementation in patients with breast cancer. In a study involving women with increased breast density, 177 participants (aged 49–65 years) with Wolfe P2/DY mammographic breast density patterns received one tablet of Promensil per day for one year. The study showed no statistically significant changes in estradiol, FSH, or luteinizing hormone (LH) levels [65]. Importantly, there were no significant differences in the breast density patterns between the treated and placebo groups. This is a positive outcome, as it is known that hormone replacement therapies (e.g., conjugated equine estrogens plus medroxyprogesterone acetate) increase mammographic density, a risk factor for breast cancer [106]. These results suggest that the consumption of red clover isoflavones by women at high risk of breast cancer may be safe, although studies have not been conducted on patients with confirmed breast cancer.

A randomized, placebo-controlled, crossover pilot study provided two Promensil tablets to 16 premenopausal women and 7 postmenopausal women for one month. It reported a nonsignificant reduction in insulin-like growth factor 1 (IGF-1) levels in perimenopausal but not postmenopausal women [78]. High serum IGF-1 levels are associated with an increased risk of breast cancer [107], making these results interesting for analysis through a larger study.

A study addressing cyclic mastalgia (breast pain) involved a placebo period of two menstrual cycles, and participants with an average pain reduction of 30% compared to baseline (i.e., a low placebo response) were then randomized and given one or two Promensil tablets (40 or 80 mg of red clover isoflavones) per day during three menstrual cycles [108]. Breast pain was significantly reduced by 44% in the 40 mg group and 31% in the 80 mg group compared to the placebo. A three-day increase in menstrual cycle length was observed in the 80 mg group compared to the placebo group. These findings imply a positive effect on breast pain in women experiencing normal hormonal fluctuations during their menstrual cycle.

A three-month study examined the endometrial effects in perimenopausal women of receiving 50 mg of red clover isoflavones daily (P-07) and found no change in the proliferative index Ki-67 in approved biopsy samples taken during the late follicular phase. There were also no changes in plasma estradiol, FSH, progesterone, or endometrial thickness [86]. Increasing the dose to 85.5 mg of isoflavones/day with Rimostil over 6 months in postmenopausal women did not result in increased endometrial thickness or breakthrough bleeding [87]. These results indicate the inability of red clover isoflavone supplements to stimulate endometrial hyperplasia in women, at least when taken over a short period.

Phytoestrogens influence the sensitivity of breast cancer cells to analogs of vitamin D3 [1]. Breast mammographic density (MBD) is considered an indicator of breast cancer progression or treatment failure [109]. This marker relates to the radiodensity of connective, epithelial, and radiolucent fatty breast tissue. Diet influences MBD, potentially affecting the level of endogenous estrogen. For example, it was observed that a low-fat, high-carbohydrate diet containing isoflavones reduced MBD [51]. Although antiestrogenic drugs like tamoxifen reduce MBD, hormone replacement therapy (HRT) increases this parameter [110]. Insulin-like growth factor-I (IGF-I) also increases the risk of developing breast cancer.

Akbaribazm et al. [8] analyzed a clinical study that was randomized, placebo-controlled, and double-blind, aiming to investigate the use of Promensil tablets daily (equivalent to 86 mg/day of total isoflavones) for 2 months in four Dutch hospitals. The study showed a reduction in IGF-I in 23 postmenopausal women who received 40 and 80 mg/day. Promensil tablets alleviated breast pain by 44% and 31% in 18 perimenopausal women over three menstrual cycles.

The presence of phytoestrogens in different parts of red clover can influence reproductive tissues and estrogen receptors. In a randomized, double-blind, placebo-controlled study, 30 postmenopausal women were treated with Promensil (equivalent to 80 mg of

red clover isoflavones) [111]. There was no significant change in their plasma levels of estradiol, FSH, or LH hormones or endometrial thickness; yet, hot flashes were reduced in 44% of the participants [68]. ER-α is expressed in breast, uterus, and ovarian tissues; ER-β is expressed in bones and blood vessels [112]. Furthermore, treatment with genistein (375 and 750 μg/g) for 21 days increased uterine weight and reduced bone loss and osteoporosis. One systematic review [45] concluded that isoflavones such as daidzein and genistein enhance the proliferation of endometrial gland cells, increase the expression of ER-β, and reduce the expression of ER-α. In a normal state, endometrial cells express higher levels of ER-α than ER-β. In an in vitro study, endometrial gland cells isolated from premenopausal and nonpregnant women in the proliferative phase after incubation with genistein (1.15 μmol/mL) and daidzein (2.4 μmol/mL) showed significant expression reduction in ER-α and an increase in ER-β mRNA [113]. The secretion of cytokines such as TNF-a and IL-1a also decreased. Finally, phytoestrogens modulate the expression of ER-α/β at the mRNA and protein levels in endometrial gland cells [114].

4.5. Osteoporosis and Ostopenia

The modern way of life increasingly eliminates movement and a healthy diet, which contribute to the reduction in bone density, resulting in osteopenia and osteoporosis [115]. Osteopenia is defined as a bone mineral density (BMD) where the T-value is between −1.0 and −2.5, and above −2.5 is the state of osteoporosis [116]. Osteopenia occurs more often in postmenopausal women due to the loss of estrogen. Osteoporosis is a chronic disease of the bone system in women, which, with its progressive course and complication, accompanied by a high degree of disability and mortality, has significant medical, economic and social consequences for both the individual and society as a whole [117]. The decrease in bone mass first affects the trabecular bone (vertebrae, ribs), which leads to kyphosis and a consequent decrease in height. Only after five or more years do the changes affect the cortical bone (neck of the femur, distal radius), which increases the possibility of fractures. The risk of osteoporosis also depends on genetic predisposition and race, eating habits, constitution, and caffeine consumption, as well as on gynecological factors, such as parity, breastfeeding, regularity of menstruation in the reproductive period, previous gynecological surgery, other diseases (hyperthyroidism, hyperparathyroidism), and taking medications such as glucocorticoids [16]. Studies have shown that taking dried red clover, an extract, or a pharmaceutical product combined with calcium can significantly slow the loss of BMD in postmenopausal women with osteoporosis [118].

In a 12-month, double-blind study with parallel design, 78 postmenopausal osteopenic women were supplemented two times a day with calcium (1200 mg/day), magnesium (550 mg/day), calcitriol (25 mg/day), and a red clover extracts rich in isoflavone aglycones and probiotics (RCE, 60 mg isoflavone aglycones/day and probiotics) or a masked placebo. RCE in combination with supplementation was more effective than supplementation alone. Over 12 months of use, RCE prevented menopause-associated decreases in BMD, normalizing bone turnover, promoting a favorable estrogen metabolite profile (2-OH:16α-OH), and stimulating equal production in postmenopausal women with osteopenia [11].

Five studies examined the effects of red clover isoflavone extracts on BMD or markers of bone turnover. Three studies reported bone preservation measured using BMD. After a 1-month placebo run-in period, a 6-month study documented increases in the proximal forearm but not in the distal forearm BMD for 25, 50, and 75 mg daily doses of red clover isoflavones in menopausal women. Another 6-month study found 4.1% and 3.0% increases in the proximal radius and ulna BMD, respectively, in postmenopausal women taking 57 mg and 85 mg of red clover isoflavones per day [6]. One-year treatment with a daily dosage of one pharmaceutical tablet significantly reduced the loss of BMD of the lumbar spine in perimenopausal women [66]. No effect was found in postmenopausal women nor was there an effect on hip BMD in either group. Possibly, red clover isoflavones prefer cortical over trabecular bone, which has yet to be confirmed.

None of the studies found effects on the levels of urinary N-telopeptide (a putative marker of bone resorption) or serum osteocalcin (a putative marker of bone formation). However, the correlation of these markers with the actual change in BMD is still unclear. The first study included perimenopausal women who took 50 mg of red clover isoflavones per day (product P-07, noncommercial, Novogen, Ltd.) for 3 months [86]. The second study used Promensil and Rimostil for 3 months in menopausal women [79]. The previously mentioned 1-year study by Atkinson et al. [65] also found no effect on bone resorption, despite positive outcomes for BMD and bone mineral content, implying that physiological levels of bone markers are inaccurate markers of BMD and bone turnover rate.

4.6. Cognitive Effects

Menopause negatively affects cognitive functions [119]. By reducing the concentration of inhibin B, it leads to a lack of negative feedback on the secretion of pituitary FSH [2,120].

From a neurobiological standpoint, the monoamine neurotransmitters serotonin, norepinephrine, and dopamine appear to modulate hot flashes and mood disorders [121]. Damage to these regulatory pathways favors the onset of depression when this dysregulation occurs within the prefrontal cortex and limbic system, which are the SNC regions involved in mood control [122]. Moreover, the deregulation of hypothalamic thermoregulatory centers affects the appearance of vasomotor symptoms. Therefore, treating hot flashes with menopausal hormone therapy (MHT) may prevent or reduce depressive symptoms in vulnerable women, rather than treating only mood disorders without controlling vasomotor symptoms [123]. A total of 40% of women reported episodes of forgetfulness in perimenopause. Women in the menopausal transition have symptoms of depression up to four times more often, which are associated with hormonal fluctuations and the presence of vasomotor symptoms [124]. Several risk factors are associated with depression, including lower socioeconomic status, stress, a previous history of depression, and a higher body mass index. A longer reproductive period is associated with a lower risk of developing depression [125].

Only one study examined the effect of red clover isoflavones on the cognitive function in postmenopausal women [126]. Thirty women over the age of 60 years received two tablets of Rimostil or a placebo for 6 months. Better results on visual–spatial intelligence tests was found in women receiving Rimostil in comparison to the placebo (12% increase vs. 3% decrease, respectively).

5. Possible Side Effects

Red clover is generally recognized as safe by the Food and Drug Administration (FDA), and most studies have found it to be well tolerated. Nevertheless, possible side effects, drug interactions, and risks for certain populations have been identified [127].

The use of red clover should be avoided in patients taking hormonal drugs, because the chemical components of red clover and hormones compete for the same hormone receptor sites and can lead to inflammation of the eyes, mouth, and penis [17].

Users of thrombolytic agents and low-molecular-weight heparin should be aware of the possibility of increased bleeding, as the plant may contain coumarins, which have anticoagulant properties. The concurrent use of red clover and contraceptives may alter the effectiveness or enhance the contraceptive's side effects. Additionally, the use of red clover with progesterone may result in reduced effectiveness. Experiments on animals conducted in vitro showed that the concomitant use of red clover and the anticancer drug tamoxifen led to the reduced effectiveness of the drugs. The plant, herbal extract, or semipurified isoflavonoids from the extract should not be used during pregnancy until further studies confirm their safety for use [128].

Additionally, no safety data on red clover among children or pregnant or breastfeeding women exist, so it should be avoided in these groups (34 trusted sources).

Finally, red clover may slow blood clotting and should be avoided in case bleeding disorders are present [129].

6. Conclusions

This narrative review provides insights into the bioactive compounds of red clover (*T. pratense* L.), particularly focused on its commercially available isoflavone extracts that have shown efficacy in the treatment of various symptoms and comorbidities in menopausal women. This review, however, did not look at other red clover extracts that are under intense research, and this is its main limitation.

Despite its well-documented traditional medicinal uses, a limited number of clinical studies tested its health effects. The majority of research has focused on red clover isoflavones, which, due to their structural similarity to endogenous 17β, estradiol can activate estrogen receptors and alleviate common menopausal symptoms. However, the other bioactive compounds isolated from red clover leaves, aglycones biochanin A and formononetin, reduce hot flashes, improve blood lipid composition, reduce atherosclerosis, maintain bone mineral density, show anticancer activity, and have cognitive effects in menopausal women. Polyphenols like daidzein isolated from red clover leaves have been shown to affect hot flashes and blood lipid composition; genistein, isolated from the leaves, stems, and aerial parts of the plant, has an effect on hot flashes, blood lipid composition, and atherosclerosis.

Despite the limited clinical data, various bioactive components isolated from red clover plant show different activities with regard to menopausal symptoms, and as such should receive more research interest with regard to developing new pharmacological strategies to improve women's health during menopause.

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