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ORIGINAL ARTICLE

Isoflavones obtained from red clover improve both dyslipidemia and menopausal symptoms in menopausal women: a prospective randomized placebo-controlled trial

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ABSTRACT

Objective: This study aimed to investigate the effects of red clover isoflavones on menopausal symptoms and the lipid profile in menopausal females.

Methods: This study included postmenopausal women with dyslipidemia. The red clover group ($n=39$) received 40 mg isoflavone red clover capsule twice daily for 6 months, while placebo ($n=36$) was 40 mg starch capsule twice daily. Data were collected at baseline, 3 months and 6 months. The Menopause Rating Scale (MRS) was applied to calculate subdimension and total scores.

Results: The two groups were similar in terms of age, MRS and lipid profile at baseline. In the red clover group, MRS scores decreased significantly at both 3 and 6 months. Similarly, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglyceride levels decreased at both 3 months and 6 months. High-density lipoprotein cholesterol increased significantly from baseline to 3 months and 6 months. Except for LDL-C and MRS urogenital score at 3 months, the improvements were significantly in favor of red clover isoflavone treatment.

Conclusions: Red clover treatment for 3–6 months demonstrated significant improvements in lipid profiles and menopausal symptoms. While promising, further research is crucial to ascertain long-term safety and recommend the use of red clover isoflavones during menopause.

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KEYWORDS
Menopause; red clover; placebo; dyslipidemia; Menopause Rating Scale

Introduction

Menopause is a natural metabolic process resulting in significant hormonal changes and is accompanied by a number of well-characterized metabolic alterations [1, 2]. Decreased estrogen causes a wide range of physical and psychosocial changes which come together to reduce quality of life [3, 4]. Alterations in hormonal balance during menopause are impactful on the plasma lipid profile, which can alter the risk of atherosclerosis and other diseases via various mechanisms [5–7].

Despite the well-established benefits of hormone replacement therapy (HRT) in premenopausal and postmenopausal women, its use in the treatment of menopausal symptoms remains controversial [1]. Concerns about the safety of hormonal drugs [8, 9] have led to an increased interest in alternative treatments such as herbal and dietary supplements (phytoestrogens) that may improve menopausal symptoms and lipid metabolism [10, 11]. Nutraceuticals and botanicals derived from plants have been used for decades to treat menopause-related health problems [12, 13]. Isoflavones are perhaps the best-known alternative compounds utilized for such purposes. Red clover (*Trifolium pratense* L., Fabaceae) is a rich source of isoflavones such as genistein, daidzein and biochanin A and B, which are similar in structure to 17β-estradiol [14]. Red clover does not have any known side effects on the uterus and breast as it exerts its effects through estrogen receptors α and β [14, 15]. Red clover extracts have antiproliferative effects on the breast and positive effects on the lipid profile and bone density [16]; however, the literature remains inconsistent [17–21]. Furthermore, an improved lipid profile has been demonstrated with administration of red clover isoflavones in animal experiments [22, 23], but outcomes are unclear in human studies [14, 24–27].

There are several design choices that could improve human studies focusing on this topic. Increasing the follow-up time and sample size appear to be feasible since many available studies have a short follow-up period [2, 24, 25] or include few subjects [24, 25, 27]. Additionally, some of these studies also do not have placebo controls [27, 28]. In this prospective placebo-controlled study, we aimed to investigate the effect of red clover isoflavones on menopausal symptoms and the lipid profile by performing 6 months of follow-up.

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Methods

Study design and ethical statement

This prospective, randomized, double-blind, placebo-controlled study was conducted between March 2022 and September 2023 in the Departments of Gynecology and Internal Medicine of Medipol University Hospital, Istanbul, Turkey. The study was approved by the local ethics committee (date: 8 March 2022, no.: E-10840098-772.02-1663) and all procedures were performed in line with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was registered under NCT06209697 (www.clinicaltrials.gov).

Enrollment process and definitions

The study included postmenopausal women who had naturally entered menopause and had applied with symptoms of menopause. Other inclusion criteria were having a history of amenorrhea for at least 12 months, being aged 45–55 years, having received a diagnosis of dyslipidemia (at present admission or within the past 3 months), not receiving treatment for dyslipidemia, absence of other chronic diseases, having a follicle stimulating hormone (FSH) level of >40 pg/ml and having a body mass index of <30 kg/m^2.

Dyslipidemia is defined as total cholesterol levels of ≥200 mg/dl, low-density lipoprotein cholesterol (LDL-C) levels of ≥130 mg/dl, high-density lipoprotein cholesterol (HDL-C) levels of ≤50 mg/dl and triglyceride levels of ≥150 mg/dl, based on the criteria set by the National Cholesterol Education Program-Adult Treatment Panel III [29]. The exclusion criteria encompassed individuals who were: currently or previously undergoing HRT (either in the past, immediately before or during the study); using any phytotherapeutic drug; diagnosed with diabetes, anemia, cardiovascular disease, musculoskeletal disease, asthma, chronic obstructive pulmonary disease, malignancy, genital or gynecological disease (excluding menopausal symptoms), neurological or psychiatric disease, or a condition involving acute or chronic inflammation; experiencing acute or chronic infection; displaying abnormal liver, kidney or thyroid function tests; engaging in smoking, alcohol consumption or drug use; suffering from sexual dysfunction (except for menopause-related reasons); unable to complete the study; or not attending 3-month or 6-month follow-up.

Study procedure and all instruments

Detailed anamnesis and full body physical examination were performed, blood pressure measurements (Omron M3 Upper Arm Blood Pressure Monitor; Japan) were made and electrocardiograms (12-Channel Edan SE-1200 Express Basic; USA) were taken at baseline for inclusion and exclusion criteria. Age information was recorded at admission. Height and weight measurements (Tanita Body Composition Analyzer, MC-780MA-N; Japan) were taken.

Randomization and medication

The subjects were divided into the red clover group (RC-G) and the placebo group (P-G). Patients were provided with slips of paper bearing numbers starting from 1, assigned based on the order of admission by the admitting physician, and directed to the appropriate nurse. The outpatient clinic nurse assigned red clover treatment to subjects with an odd-numbered slip and placebo to those with an even-numbered slip. The nurse documented the medication assigned to each patient, while the researchers and subjects remained blinded to this information. Red clover (Promensil; PharmaCare Europe Ltd, UK) and placebo (starch capsules with the same color, taste and smell as the red clover capsules) were administered orally twice a day with an interval of 12 h for a total period of 6 months. Red clover capsules contained 40 mg of standardized red clover isoflavones in each capsule (genistein [1 mg], daidzein [1 mg], biochanin A [23 mg] and biochanin B [formononetin, 15 mg]).

Laboratory analysis

All laboratory measurements were performed in our hospital's certified biochemistry laboratory, on regularly calibrated devices, according to the manufacturer's instructions. The patients' total cholesterol, LDL-C, HDL-C and triglyceride levels were measured using the enzymatic colorimetric method (Hitachi 747 autoanalyzer; Germany) from blood samples taken at 8 am after a 12-h overnight fast. Fasting blood glucose, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, free triiodothyronine and thyroxine, thyroid stimulating hormone, complete blood count, C-reactive protein and complete urinalysis were also measured with blood and urine taken at baseline. In addition to the lipid profile, these laboratory parameters were also studied after 3 months and 6 months.

Assessment of menopause symptoms

The severity of menopausal symptoms was assessed utilizing the Menopause Rating Scale (MRS). The MRS comprises 11 symptoms, and its calculation involves three subcategories and an overall score. For the MRS somatic subdimension score, the severity of symptoms such as hot flushes, heart discomfort, sleeping disorders, and joint and muscular discomfort is evaluated. The MRS psychological subscore is determined by assessing the severity of symptoms related to irritability, anxiety, and physical and mental exhaustion. The MRS urogenital subscore is calculated based on the severity of symptoms concerning sexual problems, bladder issues and dryness of the vagina. Each of the 11 symptoms is scored on a scale from 0 (no complaints) to 4 (severe symptoms), reflecting perceived severity. Consequently, the score for each subcategory is computed independently by summing the severity scores of the symptoms it encompasses. The total score is then obtained by adding these three subcategory scores [30–32]. The validity and reliability study of the Turkish version of the MRS were conducted by Metintas et al. [33].

Outcomes

The primary outcome measures were to assess the differences in MRS scores (somatic, psychological, urogenital and total score) and lipid profile (total cholesterol, LDL-C, HDL-C and triglyceride levels).

Statistical analysis

According to the study by Shakeri et al. [2], the changes in the MRS questionnaire subscales due to medication use were approximately 2.8 units (standard deviation: 3) for the vegetative somatic subscale, approximately 4.8 units (standard deviation: 4) for the psychological subscale and approximately 1.7 units (standard deviation: 2) for the urogenital subscale. In the study, with a type 1 error of $\alpha = 0.05$ and a power $(1-\beta)$ of 80%, the minimum sample sizes were determined to be 11 for the vegetative somatic subscale, eight for the psychological subscale and 13 for the urogenital subscale. The maximum calculated number was used as the sample size. Considering potential data loss (approximately 20%), a minimum of 17 participants should be included in the study. The sample size calculation was performed using MedCalc Statistical Software version 19.1 (2019, https://www.medcalc.org; MedCalc Software bv, Ostend, Belgium).

Statistical analysis was conducted using SPSS software for Windows, version 25.0 (IBM, Armonk, NY, USA). A significance level of $p < 0.05$ was considered statistically significant. Descriptive statistics were presented using the median (25th percentile–75th percentile) for continuous variables, given the non-normal distribution of the data. The Shapiro–Wilk test was employed to assess the normality of variable distributions. Between-group analyses were executed utilizing the Mann–Whitney *U* test. Within-group analyses for repeated measurements were conducted through Friedman's analysis of variance by ranks. Pairwise comparisons were adjusted using the Bonferroni correction to account for multiple comparisons.

Results

A total of 253 patients were initially assessed for eligibility, of which only 91 met the inclusion and exclusion criteria. Nine patients declined to participate voluntarily, leaving 82 patients eligible for the study. These participants were evenly distributed, with 41 individuals assigned to both the placebo and red clover groups. During the course of the study, two patients in the red clover group and five patients in the placebo group dropped out. Ultimately, the study was concluded with a total of 75 patients. The results of 39 patients in the RC-G and 36 patients in the P-G were examined. The detailed flow diagram of the study is depicted in Figure 1.

The median age of the RC-G was 48 years (46–50 years) and that of the P-G was 48 years (46–51 years). There were no significant differences between groups in terms of age ($p = 0.828$), body mass index ($p = 0.974$), lipid profile parameters, MRS subdimensions and total score at baseline. No drug-related side effects developed in any patient in either group. In terms of other laboratory tests, no significant differences were observed between the groups either at baseline or at follow-up (data not shown).

In the RC-G after 3 months, total cholesterol, LDL-C, triglyceride levels and MRS somatic, psychological, urogenital and total scores were significantly lower, and HDL-C was significantly higher compared to baseline ($p < 0.001$ for all). After 6 months, the RC-G exhibited a similar pattern, with significant reductions in total cholesterol, LDL-C, triglycerides and MRS somatic, psychological and total scores, and an increase in HDL-C compared to both baseline and the 3-month mark ($p < 0.001$ for all). Furthermore, the MRS urogenital score after 6 months was significantly lower than at baseline ($p < 0.001$). In the P-G after 3 months, MRS psychological, urogenital and total scores were significantly lower than baseline ($p < 0.001$ for all). After 6 months, the P-G displayed further improvements, with significant reductions in MRS psychological and total scores compared to both baseline and the 3-month mark ($p < 0.001$ for both). Additionally, MRS somatic and urogenital scores after 6 months were significantly lower than baseline ($p < 0.001$ for both).

The total cholesterol levels in the RC-G were significantly lower after 3 months ($p = 0.002$) and after 6 months ($p < 0.001$), with a significantly higher change between 6 months and baseline compared to the P-G ($p < 0.001$). Similarly, the LDL-C levels in the RC-G were significantly lower after 6 months ($p = 0.015$), with a significantly higher change between 6 months and baseline ($p < 0.001$). Triglyceride levels in the RC-G were significantly lower after 3 months ($p = 0.005$) and after 6 months ($p < 0.001$), with a significantly higher change between 6 months and baseline ($p < 0.001$). Additionally, HDL-C levels in the RC-G were significantly higher after 3 months ($p = 0.008$) and after 6 months ($p < 0.001$), with a significantly higher change between 6 months and baseline ($p < 0.001$).

Regarding MRS scores, the RC-G exhibited significantly lower MRS somatic scores after 3 months ($p = 0.018$) and after 6 months ($p < 0.001$), with a significantly higher change between 6 months and baseline compared to the P-G ($p < 0.001$). The RC-G also showed significantly lower MRS psychological scores after 3 months ($p = 0.018$) and after 6 months ($p < 0.001$), with a significantly higher change between 6 months and baseline ($p < 0.001$). The MRS urogenital scores in the RC-G were significantly lower after 6 months ($p = 0.033$), with a significantly higher change between 6 months and baseline ($p < 0.001$). Furthermore, the MRS total scores in the RC-G were significantly lower after 3 months ($p = 0.019$) and after 6 months ($p < 0.001$), with a significantly higher change between 6 months and baseline ($p < 0.001$) (Table 1).

Discussion

The findings of the current study demonstrated that treatment with red clover isoflavones improved menopausal symptoms and lipid levels in postmenopausal women, at both 3 months and 6 months, with notably increased impact with time.

Apart from menopausal hormonal changes, the risks associated with advanced age worsen lipid metabolism alterations, which may contribute to atherosclerosis [34]. Research has

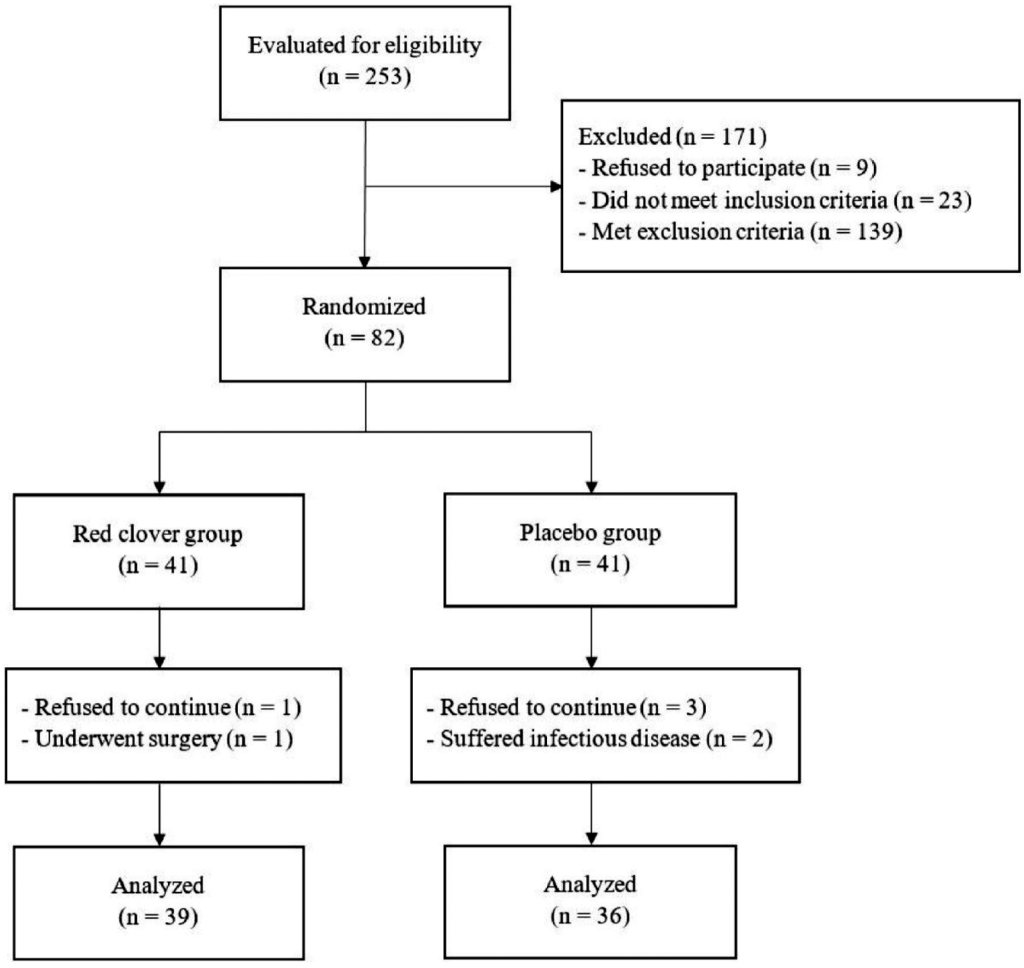

Figure 1. Flow chart of the study.

demonstrated the positive impact of HRT on lipid metabolism, as well as on the physiological and psychological changes induced by menopause [15, 35]. However, the fact that HRT increases the risk of thrombosis and cancer is a significant concern for patients and health-care providers [1]. As such, alternative therapies have been explored, including studies that have shown the effects of red clover on the lipid profile of postmenopausal and premenopausal women [14, 19, 27, 28], but with relatively inconsistent results [24, 25]. In this study, total cholesterol, LDL-C and triglyceride concentrations decreased significantly after 3 months (vs. baseline) and 6 months (vs. baseline and 3 months), while HDL-C showed a significant increase in the same comparisons. The P-G exhibited no significant change. However, with the exception of LDL-C levels after 3 months, the RC-G demonstrated significantly improved levels for all lipid parameters both at 3 months and 6 months. Similar to our results, those from a meta-analysis linked red clover to a significant increase in HDL-C along with a significant decrease in total cholesterol, LDL-C and triglycerides [26]. The results of a recent meta-analysis which included only studies with an observation period of ≥12 weeks showed that red clover was effective in reducing total cholesterol levels, but the changes in HDL-C, LDL-C and triglycerides were not as pronounced [14]. Terzic et al. showed a significant decrease in serum total cholesterol, LDL-C and triglyceride levels, and a significant increase in HDL-C levels in postmenopausal women taking red clover, compared to those who received no treatment [27, 28]. On the contrary, Nestel et al. have argued that the effect of red clover on plasma lipids is no different from placebo [24] and Campbell et al. showed that isoflavones increased HDL-C in postmenopausal women compared to placebo, but did not change total cholesterol concentrations [25]. It should be noted that both of these studies suffer from low sample size and short follow-up [24, 25]. Several factors could contribute to the lack of improvements shown in some studies, including the heterogenous characteristics of the population and the type and properties of the red clover administration (extract, specific compounds, dosage, differences in use). The duration of follow-up and variations in study designs are additional potential factors influencing the observed outcomes. Another important and potential factor is the dominant isoflavones in the red clover preparations used. This detail was also included in the meta-analysis by Kanadys et al. [14]. When preparations containing higher proportions of biochanin A and genistein were used, reductions in total cholesterol, LDL-C and triglycerides, as well as an increase in HDL-C, were reported. Conversely, when formulations containing higher proportions of formononetin and daidzein were used, the changes were not significant. However, treatments dominated by the 'formononetin/daidzein' combination were reported to have a greater effect on TC compared to the 'biochanin A/genistein' combination. The red

Table 1. Summary of demographics, laboratory measurements and MRS scores with regard to groups.

Characteristic	*Red clover (n = 39)*	*Placebo (n = 36)*	p *(between groups)*
Age (years)	48 (46–50)	48 (46–51)	0.828
Body mass index (kg/m^2)	27.3 (23.2–28.7)	27.2 (23.1–28.9)	0.974
Total cholesterol (mg/dl)			
Baseline	210 (191–227)	203.5 (192–219)	0.778
3 months	187 (172–208)[a]	203.5 (190–220)	**0.002**
6 months	164 (151–197)[a,b]	202.5 (194–219)	**<0.001**
p (within groups)	**<0.001**	0.557	
Change[c]	−38 (−58 to −24)	−1 (−5 to 4)	**<0.001**
LDL-C (mg/dl)			
Baseline	130 (118–159)	139 (119–158)	0.877
3 months	120 (109–159)[a]	133.5 (121–156)	0.128
6 months	110 (98–149)[a,b]	133 (122–155)	**0.015**
p (within groups)	**<0.001**	0.058	
Change[c]	−18 (−25 to −10)	−3 (−7 to 4)	**<0.001**
Triglyceride (mg/dl)			
Baseline	189 (167–234)	186.5 (168–252)	0.908
3 months	165 (149–187)[a]	187.5 (165–241)	**0.005**
6 months	149 (127–186)[a,b]	189 (169–230)	**<0.001**
p (within groups)	**<0.001**	0.312	
Change[c]	−48 (−85 to −22)	−0.5 (−11 to 4)	**<0.001**
HDL-C (mg/dl)			
Baseline	37 (34–39)	38.5 (35–44)	0.136
3 months	43 (40–50)[a]	40 (36–43)	**0.008**
6 months	51 (47–56)[a,b]	39.5 (38–43)	**<0.001**
p (within groups)	**<0.001**	0.852	
Change[c]	13 (8–18)	0 (−3 to 4)	**<0.001**
MRS somatic score			
Baseline	10 (6–14)	11 (7.5–15.5)	0.802
3 months	7 (4–10)[a]	10 (7–13)	**0.018**
6 months	6 (3–7)[a,b]	10 (6–11)[a]	**<0.001**
p (within groups)	**<0.001**	**<0.001**	
Change[c]	−5 (−7 to −3)	−1.5 (−3.5 to 0)	**<0.001**
MRS psychological score			
Baseline	11 (7–16)	11 (6.5–15.5)	0.855
3 months	8 (6–10)[a]	9.5 (6–13)[a]	**0.018**
6 months	5 (3–7)[a,b]	9 (5–11)[a,b]	**<0.001**
p (within groups)	**<0.001**	**<0.001**	
Change[c]	−6 (−9 to −4)	−3 (−4 to −1.5)	**<0.001**
MRS urogenital score			
Baseline	8 (4–10)	7.5 (4–10.5)	0.835
3 months	5 (2–8)[a]	6.5 (3–9)[a]	0.128
6 months	4 (2–6)[a]	6 (3–8.5)[a]	**0.033**
p (within groups)	**<0.001**	**<0.001**	
Change[c]	−3 (−4 to −2)	−1 (−2 to −1)	**<0.001**
MRS total score			
Baseline	29 (17–40)	29.5 (18–41.5)	0.815
3 months	21 (12–26)[a]	26 (15–33.5)[a]	**0.019**
6 months	15 (8–19)[a,b]	24 (14–29)[a,b]	**<0.001**
p (within groups)	**<0.001**	**<0.001**	
Change[c]	−14 (−18 to −10)	−6 (−9 to −3)	**<0.001**

[a]Significantly different from baseline.
[b]Significantly different from 3 months.
[c]Difference between 6 months and baseline: negative values represent a decrease and positive values represent an increase.
Bold values denote statistical significance at the $p < 0.05$ level. Descriptive statistics presented using the median (25th percentile–75th percentile) for continuous variables due to the non-normality of distribution. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MRS, Menopause Rating Scale.

clover preparations used in our study contain biochanin A-dominant isoflavones. This indicates that our findings support the results of the review by Kanadys et al. [14].

Kanadys et al. [14] summarized the possible mechanisms of red clover in improving dyslipidemia as follows: red clover facilitates the activation of peroxisome proliferator-activated receptors; red clover has the potential to diminish the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9); and red clover might augment liver LDL-C receptors while concurrently inhibiting endogenous cholesterol synthesis by impeding 7α-hydroxylase [36]. Although its mechanism is not fully known, as shown in our study and many previous studies, red clover isoflavones have ameliorative potential on lipid metabolism and this issue is worth investigating. The potentially positive effects of red clover on lipid metabolism need to be supported by more comprehensive studies.

Vasomotor symptoms, often followed by mood and sleep disorders, stand out as the most prevalent and distressing symptoms of menopause [2]. The effects of red clover isoflavones on menopausal symptoms, especially somatic symptoms and psychological symptoms, are uncertain. The present study indicated improvements in somatic, psychological, urogenital and overall menopausal symptoms with both 3-month and 6-month red clover treatment. Notably, with the exception of urogenital symptoms at 3 months, all symptom scores at both time points, as well as the improvement observed after 6 months, were significantly superior compared to placebo. A placebo-controlled trial reported a decrease in the

total MRS score from 20.41 to 10.08 in the red clover group and from 20.77 to 17.20 in the placebo group. This study highlighted a prominent advantage of red clover, specifically in reducing the severity of psychological symptoms [2]. However, the mentioned study did not report any improvement in the severity of urogenital symptoms, supporting prior research [19, 37]. Hidalgo et al. also showed that red clover isoflavone supplementation significantly decreased the rate and intensity of menopausal symptoms except urogenital symptoms, compared with placebo [19]. A study conducted by Lipovac et al. observed that the daily frequency of hot flushes and night sweats, as well as the overall intensity of menopausal symptoms, experienced a significant reduction following 6 months of red clover treatment in comparison to the placebo [37]. In a number of studies, isoflavones derived from red clover were effective in reducing symptoms of depression and anxiety [17] and reducing hot flush frequency compared with the placebo [1], but not in reducing other menopause symptoms [38, 39]; whereas some research has not reported a significant decrease in daily frequency of hot flushes [18] and unaltered outcomes concerning menopause symptoms [20, 21, 40]. Taken together, it remains difficult to conclude that red clover supplementation can improve all menopause symptoms. However, our study showed that red clover affected all menopause symptoms and that this effect was evident even in the third month. This could be a direct result of using isoflavone extracts and demonstrates that this issue should be further examined in future research. The number of studies investigating the effects of different types of isoflavones on vasomotor symptoms is quite limited. Tice et al. examined the effects of Rimostil tablets, which contain a higher proportion of isoflavones daidzein and formononetin, Promensil tablets, which contain a higher proportion of biochanin A and genistein, and a placebo on vasomotor symptoms. The reductions in the average daily number of hot flashes over 12 weeks were similar for the Promensil, Rimostil and placebo groups. Compared to the placebo, only Promensil was shown to reduce hot flashes more quickly [41]. Our study did not compare the effects of different types of isoflavones, but our results, using Promensil, support the findings of Tice et al. [41].

Limitations of the present study include the relatively small sample size and single centricity, which prevents broad interpretations that could apply to the population. The fact that a group receiving HRT was not included in the study limited the comparison of the success of red clover with the success of estrogen therapy. Also, despite relatively long follow-up compared to much of the literature on this topic, the long-term success and side effects of red clover have not been investigated. For this purpose, longitudinal studies with longer follow-up periods are required. Since the study population consisted of patients with dyslipidemia, a separate control group receiving conventional dyslipidemia treatment could have been valuable for comparative purposes.

Conclusions

Our results in this trial demonstrate that red clover treatment induced significant improvements in total cholesterol, LDL-C, triglyceride and HDL-C levels, as well as in all MRS scores. Therefore, the isoflavone extract of red clover appears to emerge as a potentially safe alternative to HRT in menopause. This treatment can mitigate the risk of dyslipidemia associated with menopause and can prevent or greatly reduce menopausal symptoms. However, more extensive research is warranted to explore the long-term side effects of red clover and to establish its broader acceptance in influencing lipid metabolism and alleviating menopausal symptoms.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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