



## A Comparative Study of Efficacy & Safety of Red Clover Versus Conjugated Estrogen on Vasomotor Symptoms & Sleep Patterns in Postmenopausal Women

Sangwan P<sup>1\*</sup>, Kaushal J<sup>1</sup>, Chauhan MB<sup>2</sup>, Singhal S<sup>2</sup>

<sup>1</sup> Department of Pharmacology, Pt. B.D. Sharma PGIMS, Rohtak-124001, India

<sup>2</sup> Department of Obstetrics & Gynaecology, Pt. B.D. Sharma PGIMS, Rohtak-124001, India

Address for  
Correspondance  
Sangwan P,  
[priyanka.sangwan.2004@gmail.com](mailto:priyanka.sangwan.2004@gmail.com)

### Keywords

Postmenopause;  
Hot Flash Score;  
Pittsburg Sleep  
Quality of Index;  
Red Clover;  
Conjugated  
Estrogen.

**ABSTRACT:** To compare the efficacy and safety of Red clover versus Conjugated estrogen on vasomotor symptoms and sleep patterns in postmenopausal women. This was a prospective, randomized, comparative, open label, parallel study done on 50 patients who were divided into two groups of 25 patients each and were randomized to receive treatment either with Red Clover 80mg OD (Group A) or Conjugated Estrogen 0.625mg OD (Group B) for 12 weeks. Efficacy assessment was done for vasomotor symptoms with Hot flash score and for sleep patterns with Pittsburgh sleep quality of index (PSQI) at 2, 6, 9 and 12 weeks. Safety assessment was also done by observing for the side effects due to study medications. There was statistically significant reduction in Hot flash score and PSQI in both the groups ( $p < 0.05$ ). At the end of 2, 6, 9 and 12 weeks reduction of Hot flash score in group A and group B were 9.26% vs 11.54%, 37% vs 44.23%, 66.67% vs 61.54%, 81.48 vs 88.46% respectively as compared to baseline values. At the end of 2, 6, 9 and 12 weeks reduction of PSQI in group A and group B was 6.7% vs 7.3%, 28.37% vs 29.33%, 54% vs 57.33%, 66.9% vs 72% respectively as compared to baseline values. There was more reduction in Hot flash score and PSQI with group B at the end of 12 weeks as compared to group A but the difference was not statistically significant. Safety profile of red clover was better than conjugated estrogen. Both red clover and conjugated estrogen were comparable in reduction of hot flashes and improvement of sleep disturbances whereas red clover was better tolerated. © 2015 iGlobal Research and Publishing Foundation. All rights reserved.

## INTRODUCTION

Menopause is defined as permanent cessation of menses for one year and is physiologically correlated with decline in estrogen secretion resulting from the loss of follicular function [1]. In the majority of women, menopause is a natural event occurring, on an average, at the age of 51.3 years [2]. With transition into menopause, estradiol levels fall markedly, whereas estrone levels are relatively preserved, reflecting peripheral aromatization of adrenal and ovarian androgens. FSH levels increase more than those of luteinizing hormone (LH), presumably because of the loss of inhibin, as well as estrogen feedback [3].

Hot flushes are the most common complaints of perimenopausal and menopausal women, affecting 75–85% of women. They occur with greatest frequency in the first 2 years after menopause and continue to decrease over time. Nocturnal hot flushes, the most common type, result in sleep disturbance, fatigue and depression. Sleep disturbance, a common side-effect of hot flashes, leaves many women feeling fatigued and unable to cope, which often leads to depressed mood [4].

The general approach consists of concurrent adoption of both life style modifications and drug therapy in the management of menopause. Menopausal hormone therapy, in the form of

estrogen therapy or combined estrogen-progestin therapy, is the established treatment for postmenopausal women with moderate-to-severe menopausal symptoms [5]. Only estrogen is needed for alleviating menopausal complaints, but because of an increased risk of endometrial cancer, progestin is added in women with intact uterus. Estrogen alone is given when a progestin is not tolerated or is contraindicated [6]. The general approach is to take the lowest effective dose of MHT and for the shortest time. In most cases it is best not to exceed four years of use. Conjugated estrogens are the main estrogens used in the treatment of menopausal disorders [7]. While hormone therapy is an effective treatment for menopausal symptoms, concerns about potential risks (especially cardiovascular disease, uterine and breast cancer) provide reason to consider other agents. Women who have contraindications, or are opposed to MHT, may derive benefit from the use of certain antidepressants (including venlafaxine, fluoxetine, or paroxetine), gabapentin or clonidine can be given for treatment of vasomotor symptoms and intravaginal estrogen creams or devices can be given for treatment of genitourinary symptoms [3].

Given the potential adverse events associated with MHT, other treatment options for postmenopausal symptoms have emerged over recent years, including other pharmacological agents, as well as herbal and complementary medicines [8]. Certain naturally occurring edible compounds found in plants have been shown to have some beneficial effects in relieving symptoms of menopause similar to MHT but without the appreciable adverse effects. Red clover (*Trifolium pratense/ Tripatra*) is a member of the Leguminosae family. Other names of red clover are beebread, clovone, cow Clover, meadow clover, purple Clover, wild clover and trefoil [9]. Red clover isoflavones preferentially activate the beta estrogen receptors found in the brain, bones and cardiovascular system. It shows very little activity in the alpha estrogen receptors found in breast and uterine tissue. This ensures no unfavourable effect on breast and uterus while effectively helping in managing menopausal symptoms [10]. In this study comparison of efficacy and safety of Red clover and Conjugated estrogen in postmenopausal women was made.

## **MATERIALS & METHODS**

This was a prospective, open label, randomized, comparative clinical study conducted by the Department of Pharmacology and Obstetrics & Gynaecology, Pt.B.D.Sharma PGIMS, Rohtak on 50 patients. Study was in accordance with the principles of good clinical practice (ICH-GCP) and declaration of Helsinki. An informed consent was obtained from all

patients enrolled for the study. The study was approved by Institutional Ethics Committee (IEC).

The eligible patients were randomly divided into two study groups with the help of computer generated random numbers. Each study group had 25 patients who completed the study and were subjected to as per the protocol. During the study, patients were not permitted to take any non-study drugs. The patients were screened according to following inclusion and exclusion criteria.

The inclusion criteria were postmenopausal women (permanent cessation of menses for one year) with intact uterus and those willing to give a written informed consent.

The exclusion criteria were any history of unexplained vaginal bleeding, history of endometrial cancer, breast cancer, patients on hormonal replacement therapy, history of venous thromboembolism, myocardial infarction, coronary heart disease (CHD), stroke or transient ischemic attack, diabetes mellitus, uncontrolled hypertension, hypertriglyceridemia (>400 mg/dl), active gallbladder disease, active liver disease and those who refused to come for regular follow ups.

Patients fulfilling the above criteria were divided into two groups. Each study group had 25 patients and received one of the following treatments orally: group A received Red Clover 80mg once in a day and group B received Conjugated Estrogen 0.625mg once daily. A provision was made for escape treatment for those patients whose symptoms were not adequately controlled with Red clover. A provision was made to treat those patients with the conjugated estrogen as per the standard treatment guidelines and to drop them from the study.

Clinical assessment was carried out in all the patients in terms of efficacy of the treatment along with safety estimation which was done at 0, 2, 6, 9 and 12 weeks for the following parameters. End points of efficacy were Hot flash score for vasomotor symptoms and Pittsburgh sleep quality index (PSQI) for sleep quality. Hot flash score was calculated as follows: Hot Flash Score = (number of mild hot flashes in a day x 1) + (2 x number of moderate hot flashes) + (3 x number of severe hot flashes).

The PSQI is a well validated questionnaire that broadly measures sleep quality. It is commonly used in both research and clinical practice. It consists of nine questions regarding various aspects of sleep. These questions include the usual time of sleep, time taken to fall asleep, time at which person gets up in the morning, any nocturnal awakenings and the reason of these awakenings, any medication to improve sleep,

difficulty in carrying out the day to day procedures and finally how the patient subjectively rates this sleep. These are translated into seven components, which are then combined to yield a global, composite score (range from 0 to 21). A global sum of five or more indicates a poor sleep. Safety assessment was done by observing side effects and doing relevant investigations.

Primary objective was to compare the clinical efficacy of two study regimens. Data was expressed as Mean  $\pm$  SEM. Both intragroup and intergroup statistical analysis was done. Intragroup analysis for repeated measures was done using ANOVA for parametric data. Intergroup analysis was done using unpaired 't' test for parametric data. Categorical data like incidence of adverse events in both the groups was also analysed. A p-value  $<0.05$  was considered as statistically significant.

## **RESULTS & DISCUSSION**

A total of 71 patients with postmenopausal symptoms were screened for this study. Out of this, 8 patients were excluded, as 5 patients did not fulfill the predefined inclusion criteria of the study and 3 were not willing to give informed consent. Rest of the 63 patients, enrolled in the study were randomized with the help of computer generated random numbers and were allocated to either of the two treatment groups. Patient in group A received Red clover 80 mg OD while group B received Conjugated estrogen 0.625 mg OD for 12 weeks. Of the 63 patients enrolled in the study, 33 were allocated to Red clover 80 mg OD group and 30 allocated to conjugated estrogen 0.625 mg OD group. 8 patients in group A and 5 patients in group B were lost to follow-up and were dropped from the study and the remaining 25 patients in either group completed the treatment successfully. The baseline characteristics of the patients are tabulated in table-1. There was no statistically difference in the baseline characteristics in both the groups.

The mean age of the patients in years was  $50.84 \pm 1.042$  and  $51.96 \pm .99$  (Mean $\pm$ SEM) in group A and group B respectively. The difference in the age between the two groups was not statistically significant ( $p=0.441$ ). (Table-1)

The hot flash score was recorded in all the patients of either group before drug administration (baseline) and at end of 2, 6, 9 and 12 weeks.(Table-2 and fig-1)On intragroup analysis, there was statistically significant reduction in hot flash score at 2, 6, 9 and 12 weeks compared to baseline values in both group A & B. In group A there was 9.26% reduction in hot flash score whereas, reduction in group B was around 11.54% at end of 2 weeks compared to their baseline values ( $p<0.05$ ).

At the end of 6, 9 and 12 weeks reduction in group A and group B were 37% vs 44.23%, 66.67% vs 61.54%, 81.48 vs 88.46% respectively as compared to baseline values. On intergroup analysis, at the end of 12 weeks there was more reduction in hot flash score with group B as compared to group A but the difference was not statistically significant.

Another parameter, PSQI was recorded in all the patients of either group before drug administration (baseline) and at end of 2, 6, 9 and 12 weeks. On intragroup analysis, there was statistically significant reduction in PSQI at 2, 6, 9 and 12 weeks compared to baseline values in both group A & B. In group A there was 6.7% reduction in PSQI whereas, reduction in group B was around 7.3% at end of 2 weeks compared to their baseline values ( $p<0.001$ ). At the end of 6, 9 and 12 weeks reduction in group A and group B was 28.37% vs 29.33%, 54% vs 57.33%, 66.9% vs 72% respectively as compared to baseline values. On intergroup analysis, at the end of 2, 6, 9 and 12 weeks there was more reduction in PSQI with group B as compared to group A but the difference was not statistically significant. (Table-3 and fig-2).

All the patients responded to study medications, so escape treatment was not required in any of the patients of either group.

Safety assessment was done for the side effects like nausea, myalgia, headache, mastalgia, vaginal bleeding, rashes, weight gain, diarrhoea, insomnia and dizziness at the end of week 2, 6, 9 and 12 following drug administration. The frequency of adverse drug reactions (ADRs) as shown in table-4, was 11(44%) in group B vs 8(32%) in group A ( $p>0.05$ ). Thus, no statistically significant difference was observed between the two groups regarding the incidence of adverse effects. The incidence of reported ADRs was as follows in both the groups. Nausea, headache, rashes, dizziness and insomnia were observed in one patient each (4%) in both groups. 3 patients (12%) in group A and 1 patient (4%) in group B reported diarrhoea. Mastalgia was observed in three patients (12%) in group B whereas in none of the patients in group A. In addition, myalgia was observed in one patient (4%) in group B whereas in none of the patients in group A. No new adverse reactions were reported in both the groups. None of the patients withdrew from the study due to ADRs. This shows that all treatments regimens were well tolerated with minor ADRs.

Safety assessment was done at baseline and subsequently at 2, 6, 9 and 12 weeks after starting the treatment for hematological parameters (hemoglobin, total and differential count). Biochemical parameter (lipid profile) assessment and

ultrasonography of uterus, adnexae and breasts were done at baseline and week 12. On intragroup analysis and intergroup analysis, there was no statistically significant difference in the hemoglobin, total leucocyte count, differential leucocyte count in both the groups and between the groups respectively, over a period of 12 weeks.

On intragroup analysis as shown in table-5, both the groups were observed to be safe for the lipid profile. Moreover, both the groups led to rather reduction in total cholesterol, LDL-cholesterol and increase in HDL-cholesterol and this was statistically significant at the end of 12 weeks compared to their baseline values. The improvement in the lipid profile observed was as follows in group A and B respectively: Total cholesterol decreased by 8.92 & 5.96, LDL-cholesterol 10.88 & 7.83 and HDL-cholesterol increased by 5.04 & 2.40. Triglycerides in group A decreased by 3.88 while in group B increased by 0.88. On intergroup analysis, better results were observed in group A as compared to group B i.e. more reduction in total cholesterol, triglycerides and improvement in HDL-cholesterol were observed over a period of 12 weeks and this difference was statistically significant with p values of .044, .046 and .008 respectively. No statistically significant difference was observed in between the groups in LDL-cholesterol and VLDL-cholesterol.

Ultrasonography of uterus, adnexae and breasts were done in all the patients at baseline and at the end of 12 weeks to detect any drug induced abnormalities. None of the patients had any abnormality in USG of uterus, adnexae and breasts at baseline and even after completion of the study i.e. after 12 weeks, USG was normal in both the groups. Thus both drugs were safe for uterus adnexae and breasts.

Menopause is defined as 'the permanent cessation of menstruation for one year resulting from loss of ovarian follicular activity' [1]. Natural menopause is not a singular event but a transition lasting for an average period of 3.8 years [11]. This phase of ageing process during which a woman passes from reproductive to non-reproductive stage is known as climacteric. It covers 5-10 years on either side of menopause. It can be further divided into 3 phases, these are premenopause, perimenopause and finally postmenopause. Premenopause refers to the period prior to menopause, perimenopause is the period around menopause and postmenopause is the period after menopause [1].

Treatment is indicated if menopausal symptoms interfere with a woman's daily functioning and quality of life. Recently in the 2013 update of the International Menopause Society (IMS) recommendations, the term Menopausal hormone therapy

(MHT) has been used to cover therapies including estrogens, progestogens and combined therapies. Estrogen alone is given when a progestin is not tolerated or is contraindicated [6]. Conjugated estrogens are the main estrogens used in the treatment of menopausal disorders [12]. Given the potential adverse events associated with MHT, other treatment options for postmenopausal symptoms have emerged over recent years, including other pharmacological agents, as well as herbal and complementary medicines. Among herbal products one is red clover which is a natural SERM with preferential action on estrogen receptor beta (mostly present in brain, bone, and heart) while little activity towards estrogen receptor alpha (mostly present in breast and uterus). This ensures no unfavorable effect on breast and uterus while effectively helping in managing menopausal symptoms [13]. Red clover has good safety profile, no significant side effects are mentioned in studied literature [14].

Some studies have compared the efficacy and safety of conjugated estrogen with placebo and red clover with placebo. To the best of our knowledge, no such study involving comparison of conjugated estrogen and red clover has been done worldwide. Hence, the present study was therefore done to compare the efficacy for treatment of vasomotor symptoms and sleep quality in postmenopausal women. We also compared safety of red clover versus conjugated estrogen in postmenopausal women. This was a prospective, open label, randomized, comparative clinical study. The present study revealed that both the groups led to statistically significant reduction ( $p < 0.05$ ) in hot flash score and Pittsburgh sleep quality of index over a period of 12 weeks as compared to baseline values.

Analysis of the hot flash score in the present study showed that both the groups were comparable regarding the reduction of hot flash score and there was no statistically significant difference. Follow up visits showed an improvement in percentage reduction of mean hot flash score in both the groups. Improvement in hot flash score was seen as early as 2 weeks of treatment in red clover group (9.26%) as compared to their baseline values ( $p < 0.05$ ) and this reduction was continuous over 12 weeks (81.48%). In the present study, fall in mean hot flash score was also seen in the conjugated estrogen group in the week 2 (11.5%) which was continuous till week 12 i.e. 88.46%. Although exact similar studies were not available in which similar treatment groups were compared for observing the effects on hot flash score. However after literature search, we could get the studies in which red clover and conjugated estrogens were compared with placebo. Three randomized controlled trials of red clover reported beneficial effects over placebo [15-17]. In a double-

blind placebo-controlled study done by Van de Weijer et al, the effect of 80 mg red clover isoflavones and placebo on vasomotor symptoms in postmenopausal women was evaluated. There was no significant change in hot flushes in the placebo group whereas it decreased by 44% in the red clover isoflavones group by week 12. There was a significant difference between the placebo and treatment group in the median percentage reduction of hot flushes from baseline at weeks 8 and 12 [15]. The findings of our study are similar to this study as red clover led to statistically significant reduction in hot flash score as in the above mentioned study. However the reduction was even better than the above quoted study. In another study, Jeri et al compared 40 mg/day isoflavones (Red clover) with placebo for 16 weeks in a small sample (N = 30) of women in Peru. A significant ( $P < 0.001$ ) reduction in hot flash frequency (48.5%) and severity was reported in the active group compared with the placebo group, who experienced a small reduction in frequency (10.5%) and no change in hot flash severity [16]. The findings of our study are similar to this study as red clover led to statistically significant reduction in hot flash score as in the above mentioned study. However the reduction was even better than the above quoted study. The reason could be due to the fact that higher dose i.e. 80 mg was used in our study as compared to 40 mg in the above mentioned study. In another study, Lipovac et al evaluated the effect of red clover (80 mg) on vasomotor symptoms and compared with placebo for a 90-day period. After a washout period of 7 days, medication was crossed over and taken for 90 days more. Daily hot flush was measured at baseline, 90, 97 and 187 days. Daily hot flush frequency decreased after red clover phase in Group A to a 73.5% average decrement. This decrement was significantly higher than those observed for Group B after placebo phase (8.2%) [18]. The findings of our study are similar to this study as red clover led to statistically significant reduction in hot flash score as in the above mentioned study and the reduction was also quite similar as in the above mentioned study. i.e. 81.48% reduction in our study vs 73.5% in above study over a period of 12 weeks (90 days).

In our study, on intragroup analysis, there was statistically significant reduction in PSQI at 2, 6, 9 and 12 weeks compared to baseline values in both group A & B. In group A there was 7.3% reduction in PSQI whereas, reduction in group B was around 6.7% at end of 2 weeks compared to their baseline values ( $p < 0.001$ ). At the end of 6, 9 and 12 weeks reduction in group A and group B was 28.37% vs 29.33%, 54% vs 57.33%, 66.9% vs 72% respectively as compared to baseline values. There was more reduction in PSQI with group B as compared to group A but the difference was statistically not significant. Although no exact similar studies were

available in which red clover and conjugated estrogens were compared for observing the effect on PSQI. Some studies were available where other tools were used for assessment of quality of sleep for eg. polysomnographic changes, sleep electroencephalogram etc. In a single-center, double-blind, placebo-controlled pilot study, patients who received 0.625 mg daily of synthetic conjugated estrogens, the polysomnographic change in sleep measures did not reach statistical significance, but the data suggests an overall improvement in sleep quality in the treatment group [19]. As the parameter used for assessment of sleep quality in our study is different from above mentioned study, so no exact comparison could be made with this study but inference could be drawn that conjugated estrogen in our study improved sleep in postmenopausal patients as in above mentioned study. Thus the findings in our study are similar to above mentioned study in the view of improvement in sleep quality. In another study, a sleep electroencephalogram was recorded in 11 postmenopausal women with and without estrogen administered by skin patch (50  $\mu\text{g}$  of estradiol per day). Estrogen enhanced rapid-eye-movement sleep ( $50 \pm 4$  vs  $39 \pm 5$  minutes,  $P < .05$ ) and reduced time awake ( $12 \pm 5$  vs  $20 \pm 6$  minutes,  $P < .05$ ) during the first 2 sleep cycles. The normal decrease in slow-wave sleep and delta activity from the first to the second cycle (in percentage from the first cycle) was restored by estrogen ( $-56\% \pm 9\%$  vs  $-5\% \pm 14\%$  and  $-20\% \pm 6\%$  vs  $-2\% \pm 5\%$ ;  $P < .05$ , respectively) [20]. The findings of our study are similar to this study as estrogen improved sleep quality as in above mentioned study. In one study done by Geller et al, which was a randomized, four-arm, double-blind clinical trial of standardized black cohosh, red clover, placebo and 0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate (CEE/MPA;  $n = 89$ ) were given a modified version of the Pittsburgh Sleep Quality Index to assess sleep. No significant differences were observed between any of the treatment groups and placebo at any of the time points measured [21]. The findings of our study are quite similar to above mentioned study in view of the fact that although sleep quality was improved with both the drugs i.e. red clover and conjugated estrogen, however no statistically significant difference was observed between the two groups as far as reduction in PSQI was concerned.

In our study, the incidence of reported ADRs was 11 (44%) in group B i.e. conjugated estrogen Vs 8 (32%) in group A i.e. red clover. So red clover was found to be safer although difference was not statistically significant. Tempfer et al, compared the incidence of side effects between participants who took phytoestrogens (isoflavones) with those who took placebo, they found it to be nearly the same: 36.7 percent in the phytoestrogen group and 38 percent in the placebo group

[22]. However, in our study incidence with red clover was less i.e. 32%. This shows that red clover isoflavones are quite safe. In a study done by Booth et al, it was reported that red clover is generally well tolerated but can cause minor adverse effects, some occurring at doses as low as 40 mg per day. Adverse effects include: headache, myalgia, arthralgia, nausea, diarrhoea, breast tenderness, swollen neck glands, dizziness, vertigo, tremor, hypertension, acne and rash [23]. The side effects in our study are less as compared to the above mentioned study. This shows that red clover isoflavones are quite safe. In a study done by Ingram et al, the administration of Red clover isoflavones has also been correlated with a significant decrease in mastalgia symptoms. In placebo group there was no change in mastalgia symptoms [24]. In our study also, there was no incidence of mastalgia as compared to conjugated estrogens group in which mastalgia was reported in three patients. This shows that red clover is safe for the breasts in postmenopausal women.

Nausea and vomiting are an initial reaction to estrogen therapy in some women, but these effects may disappear with time and may be minimized by taking estrogens with food or just before sleep. Fullness and tenderness of the breasts and edema may occur but sometimes can be diminished by lowering the dose [25]. In our study, also almost similar side effects were observed with conjugated estrogen i.e. nausea, mastalgia, headache. Other side effects observed were myalgia, rashes, diarrhoea and insomnia.

Intragroup and intergroup analysis showed no statistically significant difference in the hematological parameters in both the groups over a period of 12 weeks. To the best of our knowledge, we couldn't find the similar study in which similar treatment regimens were assessed for their effect on hematological parameters. In a study done by Knight et al, there was no change in biochemical and haematological parameters in red clover group [26]. This was supported by Hale et al who also showed that there was no change in hematological parameters [27]. Our study showed similar results as in above mentioned study, as there was no change in hematological parameters.

In our study, both the groups led to reduction in total cholesterol, LDL-cholesterol and increase in HDL-cholesterol and this was statistically significant at the end of 12 weeks compared to their baseline values. On comparing both groups, better results were observed in red clover as compared to conjugated estrogens i.e. more reduction in total cholesterol, triglycerides and improvement in HDL-cholesterol were observed over a period of 12 weeks and this difference was statistically significant with p values of .044, .046 and .008

respectively. Triglycerides were rather increased in conjugated estrogens group in comparison to the red clover group in which there was decrease in triglycerides levels. No statistically significant difference was observed in between the groups in LDL-cholesterol and VLDL-cholesterol.

In a 12 week, double-blind, randomized trial in 100 Peruvian women to investigate the effects of red clover (40 mg) versus placebo on postmenopausal women with borderline hyperlipidemia, it was shown that there was improvement in total cholesterol, LDL and HDL ratio with red clover [28]. The results of our study are quite similar to above mentioned study in view of the facts that improvement in lipid profile was observed as depicted in the above study. This shows that red clover is safe as far as the effects on lipid profile are concerned.

In another study done by Terzic et al, in women with borderline hyperlipidaemia, a significant increase in high-density lipoprotein cholesterol and a reduction in apolipoprotein were observed from baseline to 12 weeks [29]. The results of our study are quite similar to above mentioned study in view of the facts that improvement in lipid profile was observed as depicted in the above study. This shows that red clover is safe as far as the effects on lipid profile are concerned.

Campbell et al, found that 1-month supplementation with red clover isoflavones (86 mg/day red clover-derived isoflavones) increased HDL in postmenopausal women compared to placebo (P=0.02) but did not alter either cholesterol or triacylglycerol concentrations, and had no effect on antioxidant status [30]. The results of our study are quite similar to above mentioned study in view of the facts that improvement in lipid profile (improvement in HDL) was observed as depicted in the above study. This shows that red clover is safe for the lipid profile.

In a study done by Hidalgo et al, 60 postmenopausal women received either a red clover isoflavone supplement (80 mg/day) or placebo for 90 days. Subsequently, after a 7-day washout period, subjects switched to receive the opposite treatment for a further 90 days. In the red clover group, mean total cholesterol, low-density lipoprotein-cholesterol and triglyceride levels also decreased; however, only the latter was significantly lower compared with placebo [17]. The findings in our study are similar to above mentioned study as total cholesterol, LDL cholesterol and triglycerides were decreased with red clover as in above mentioned study.

Estrogens slightly elevate serum triglycerides and slightly reduce total serum cholesterol levels. More important, they

increase high-density lipoprotein (HDL) levels and decrease the levels of low-density lipoprotein (LDL) and lipoprotein A (LPA) [25]. In our study, also almost similar results were observed with conjugated estrogen i.e. decrease in levels of total cholesterol, LDL, increase in HDL and a slight increase in triglycerides. This shows that conjugated estrogen is safe for lipid profile.

In our study, none of the patients had any abnormality in USG of uterus and adnexae at baseline and even after completion of the study i.e. after 12 weeks. Normal USG suggested that drugs were safe for uterus and adnexae.

In a study done by Woods et al, A total of 29 women took part in a crossover trial with two eight-week phases. No increase in endometrial thickness measured by doppler ultrasonography was observed in red clover or placebo [31]. The findings of our study are quite similar to this study as red clover was found to be safe for the uterus.

In another study done by Baber et al, Vaginal ultrasound was performed in 43 postmenopausal women after 12 weeks dosing at 40 mg per day. There was no change in the endometrial thickness of the uterus from baseline measures [32]. The findings of our study are quite similar to this study as red clover was found to be safe for the endometrium.

To the best of our knowledge, no study was available in which effects of conjugated estrogen were observed on uterus and adnexae.

In our study, USG of breasts was done in all the patients at baseline and at the end of 12 weeks for detection of any abnormalities caused by drugs. None of the patients had any abnormality in USG of breasts at baseline and at the end of 12 weeks. Hence, both drugs were safe for breasts.

A study was done by Atkinson et al, a total of 177 women completed the study, in which comparison of red clover was done with placebo. Mammographic breast density decreased in both the groups but the difference between the treatment

and placebo was not statistically significant [32]. The findings of our study are quite similar to this study as red clover did not produce any adverse effects on the breasts. This shows that red clover is safe for the breasts in postmenopausal women.

Powles et al had done a study on healthy women aged 35–70 years (n = 401) with at least one first-degree relative with breast cancer, who received red clover isoflavones or placebo for three years in a randomized, double-blind, placebo controlled trial. No significant differences in breast density detected between those taking red clover isoflavones and placebo [33]. The findings of our study also show that red clover did not produce any adverse effects on the breasts. This shows that red clover is safe for the breasts. To the best of our knowledge, no study was available in which effects of conjugated estrogen were observed on breasts.

**Table 1. Comparison of study population characteristics in both the groups**

<b>Variables</b>	<b>Group A (n=25)</b>	<b>Group B (n=25)</b>	<b>'p' value</b>
<b>Age in years</b>	50.84 ± 1.04	51.96 ± .99	.441
<b>Weight (Kgs)</b>	62.36 ± 1.19	60.44 ± 1.46	.315
<b>Marital Status</b>			
<b>Married</b>	24	25	-
<b>Widow</b>	1	0	-
<b>Education</b>			
<b>Literate</b>	17	18	-
<b>Illiterate</b>	8	7	-
<b>History of drug allergy</b>	0	0	-

- Age and weight are expressed as Mean ± SEM
- Group A: Red Clover 80 mg
- Group B: Conjugated Estrogens 0.625 mg

Table 2. Comparison of changes in hot flash score in both the groups

Hot Flash score	Red Clover ( Group A)		Conjugated Estrogen ( Group B)		p value ( inter-group)
	Mean ± SD	%reduction from baseline	Mean ± SD	%reduction from baseline	
Baseline	2.16 ± .688	0	2.08 ± .702	0	.686
2 weeks	1.96 ± .676*	9.26	1.84 ± .747*	11.54	.554
6 weeks	1.36 ± .490*	37	1.16 ± .473*	44.23	.148
9 weeks	.72 ± .614*	66.67	.80 ± .500*	61.54	.616
12 weeks	.40 ± .500*	81.48	.24 ± .436*	88.46	.234

**INTRAGROUP ANALYSIS:**

\* Comparison of values at end of week 2, 6, 9 and 12 with baseline values are statistically significant. (p<0.05).

**INTERGROUP ANALYSIS:**

Comparison of values between group A and B are not statistically significant. (p>0.05)]

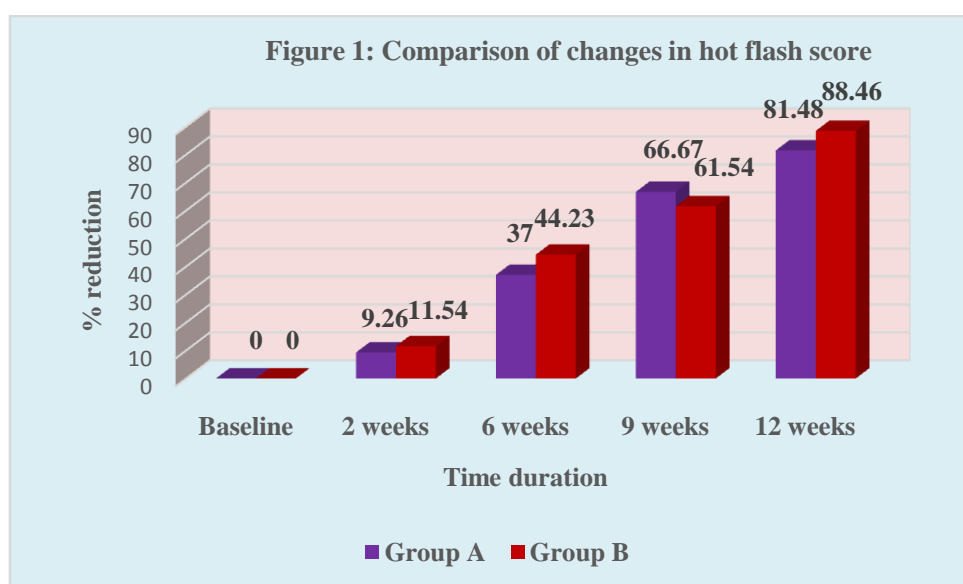


Table 3. Comparison of changes in psqi in both the groups

PSQI	Red Clover ( Group A)		Conjugated Estrogen ( Group B)		p value ( inter-group)
	Mean ± SD	%reduction from baseline	Mean ± SD	%reduction from baseline	
Baseline	2.96 ± 2.922	-	3 ± 2.930	-	.962
2 weeks	2.76 ± 2.570*	6.7%	2.78 ± 2.896*	7.3%	.602
6 weeks	2.12 ± 1.965*	28.37%	2.12 ± 1.965*	29.33%	.832
9 weeks	1.36 ± 1.381*	54%	1.28 ± 1.966*	57.33%	.830
12 weeks	.98 ± .958*	66.9%	.84 ± .898*	72%	.306

**INTRAGROUP ANALYSIS:**

\* Comparison of values at end of week 2, 6, 9 and 12 with baseline values are statistically significant (p<0.05).

**INTERGROUP ANALYSIS:**

Comparison of values between group A and B are not statistically significant (p>0.05)



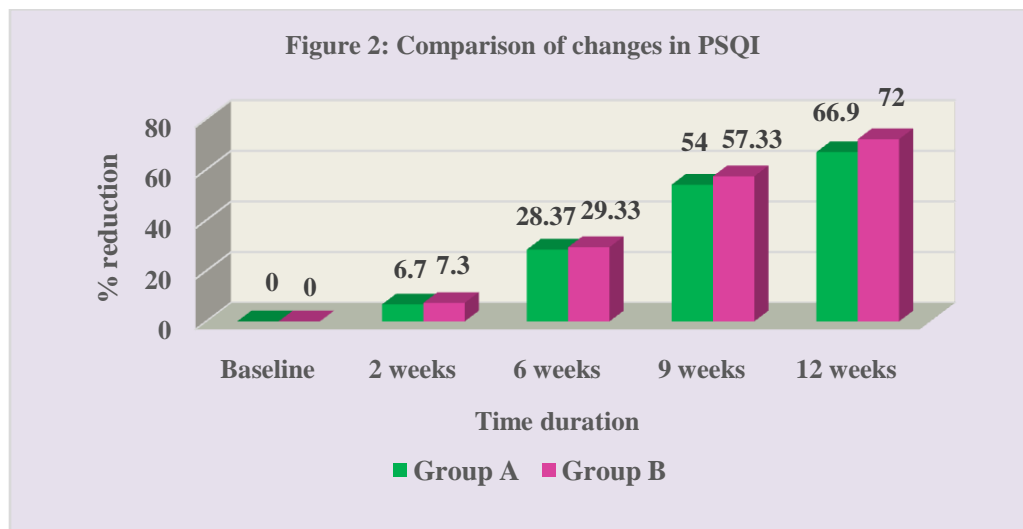


Table 4. Incidence of adverse drug reactions

Adverse Drug Reactions (ADRs)	Group A n=25(%)	Group B n=25(%)
Nausea	1 (4)	1 (4)
Myalgia	0	1 (4)
Headache	1 (4)	1 (4)
Mastalgia	0	3 (12)
Vaginal bleeding	0	0
Rashes	1 (4)	1 (4)
Weight gain	0	0
Diarrhoea	3 (12)	1 (4)
Insomnia	1 (4)	1 (4)
Dizziness	1 (4)	2 (8)

- All values are expressed as number of patients (Percentage)
- Group A: Red Clover 80 mg
- Group B: Conjugated Estrogen 0.625 mg

Table 5. Comparison of biochemical parameters in both the groups

Parameters	Baseline		12 Weeks	
	Grp A	Grp B	Grp A	Grp B
<b>Lipid profile</b>				
<b>Total cholesterol</b>	188.48 ± 10.38	190.48 ± 10.13	179.56 ± 8.40* <sup>^</sup>	184.52 ± 8.54* <sup>^</sup>
<b>Triglycerides</b>	128.52 ± 13.33	130.92 ± 13.51	124.64 ± 11.74* <sup>^</sup>	131.80 ± 12.89 <sup>^</sup>
<b>HDL-C</b>	40.64 ± 2.70	40.72 ± 2.52	45.68 ± 3.64* <sup>^</sup>	43.12 ± 2.84* <sup>^</sup>
<b>LDL-C</b>	120.36 ± 4.20	124.92 ± 10.67	109.48 ± 12.23*	116.09 ± 2.47*
<b>VLDL-C</b>	25.84 ± 2.62	25.44 ± 2.93	25.12 ± 2.35	25.80 ± 2.84

- All values are expressed as Mean ± SD
- Group A: Red Clover 80 mg
- Group B: Conjugated Estrogen 0.625 mg

**INTRAGROUP ANALYSIS:**

\* Comparison of values at end of week 12 with baseline values are statistically significant ( $p < 0.05$ ).

**INTERGROUP ANALYSIS:**

<sup>^</sup> Comparison of values between group A and B which are statistically significant ( $p < 0.05$ ).

## CONCLUSION

Both the treatment groups i.e. red clover and conjugated estrogen were found to be safe and efficacious in postmenopausal patients as both led to reduction in hot flash score and pittsburgh sleep quality index. On comparing the above mentioned treatment groups, the results of conjugated estrogen were comparable with red clover. However, red clover was better tolerated.

## REFERENCES

- Shifren JL, Schiff I. Menopause. In: Berek JS, editor. Berek and Novak's Gynaecology. 15<sup>th</sup> ed. Philadelphia: Lippincott Williams & Wilkins; 2012. p. 1233-49.
- Dratva J, Gomez Real F, Schindler C, Ackermann-Liebrich U, Gerbase MW, Probst-Hensch NM, et al. Is age at menopause increasing across Europe? *Menopause*. 2009;16(2):385-94.
- Cachermann J, Jameson JL. The Menopause transition and postmenopausal hormone therapy. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors.
- Fuh JL, Wang SJ, Lu SR, Juang KD, Chiu LM. The Kinmen women-health investigation (KIWI): a menopausal study of a population aged 40-54. *Maturitas*. 2001 Aug 25;39(2):117-24.
- North American Menopause Society. The 2012 hormone therapy position statement of: The North American Menopause Society. *Menopause*. 2012 Mar;19(3):257-71.
- De Villiers TJ, Pines A, Panay N, Gambacciani M, Archer DF, Baber RJ, et al. Updated 2013 International Menopause Society recommendations on menopausal hormone therapy and preventive strategies for midlife health. *Climacteric*. 2013;16:316-37.
- Jaakkola S, Lyytinen HK, Dyba T, Ylikorkala O, Pukkala E. Endometrial cancer associated with various forms of postmenopausal hormone therapy: a case-control study. *International Journal of Cancer*. 2011;128:1644-51.
- Nelson HD, Vesco KK, Haney E, Fu R, Nedrow A, Miller J, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. *JAMA*. 2006;295:2057-71.
- British Herbal Pharmacopoeia. Medicinal Plants of USA. Krochmal A and Krochmal C. New York: New York Times Book Co. 1990;1.
- Covse JF, Lindzey J, G Randien K, Gustafsson JA, Korach KS. Tissue Distribution and Quantitative analysis of Estrogen Receptor Alpha and Estrogen Receptor Beta. *Endocrinol*. 1997;138(11):64613-21.
- Avis NE, McKinlay SM. The Massachusetts Women's Health Study: an epidemiologic investigation of the menopause. *J Am Med Womens Assoc*. 1995;50(2):45-9.
- Bulun SE, Adashi EY. The physiology & pathology of the female reproductive axis. In: Larsen, Kronenberg, Melmed, and Polonsky, editors. Williams's Textbook of Endocrinology. Philadelphia, PA: Saunders; 2003. p. 587-684.
- Nikov GN. *Environ Health Perspect*. 2000;108:867-72.
- Lipovac M, Chedraui P, Gruenhut C, Gocan A, Kurz C, Neuber B et al. Effect of Red Clover Isoflavones over Skin, Appendages, and Mucosal Status in Postmenopausal Women. *Obstet Gynecol Int*. Nov 2011;2011:949302.
- Van de Weijer PH, Barentsen R. Isoflavones from red clover (Promensil) significantly reduce menopausal hot flush symptoms compared with placebo. *Maturitas*. 2002;42(3):187-93.
- Jeri AR. The use of an Isoflavone supplement to relieve hot flushes. *The Female Patient*. 2002;27:35-7.
- Hidalgo LA, Chedraui PA, Morocho N, Ross S, Miguel GS. The effect of red clover isoflavones on menopausal symptoms, lipid and vaginal cytology in menopausal women: A randomised, double-blind, placebo-controlled study. *Gynecol Endocrinol*. 2005; 21(5):257-64.
- Lipovac M, Chedraui P, Gruenhut C, Gocan A, Stammeler M, Imhof M. The effect of red clover isoflavone supplementation over vasomotor and menopausal symptoms in postmenopausal women. *Gynecol Endocrinol*. 2011:1-5.
- Gambacciani M, Ciaponi M, Cappagli B, Monteleone P, Benussi C, Bevilacqua G, et al. Effects of low-dose, continuous combined hormone replacement therapy on sleep in symptomatic postmenopausal women. *Maturitas*. 2005;50:91-7.
- Irina A, Gunter K, Steiger A. Modulation of the sleep electroencephalogram by estrogen replacement in postmenopausal women. *Amer J Obstet Gynecol*. 2000;182(2):277-82.
- Geller SE, Shulman LP, van Breemen RB, Banuvar S, Zhou Y, Epstein G, et al. Safety and Efficacy of Black Cohosh and Red Clover for the Management of Vasomotor Symptoms: A Randomized Controlled Trial. *Menopause*. 2009 Nov-Dec;16(6): 1156-66.
- Tempfer CB, Froese G, Heinze G. Side effects of phytoestrogens: a meta-analysis of randomized trials. *Am J Med*. 2009 Oct;122(10):939-46.
- Booth N, Overk C, Yao P, Totura S, Deng Y, Hedayat AS, et al. Seasonal Variation of Red Clover (*Trifolium pratense* L., Fabaceae) Isoflavones and Estrogenic Activity. *Journal of Agricultural and Food Chemistry*. 2006;54:1277-82.
- Ingram DM, Hickling C, West L, Mahe U, Dunbar PM. A double blind randomised controlled trial of isoflavones in the treatment of cyclical mastalgia. *Breast*. 2002;11(2):170-4.
- Levin ER, Hammes SR. Estrogens and Progestins. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's The pharmacological basis of therapeutics. 12<sup>th</sup> ed. New York: Mc Graw Hill; 2011. p. 1163-94.
- Knight D, Howes J, and Eden J. The effect of Promensil, an isoflavone extract, on menopausal symptoms. *Climacteric*. 1999;2:79-84.
- Hale GE, Hughes CL, Robboy SJ, Agarwal SK, Bievre M. A double-blind randomized study on the effects of red

- clover isoflavones on the endometrium. *Menopause*. 2001;8(5):338-46.
28. Jeri A. Effects of isoflavone phytoestrogens on lipid profile in postmenopausal Peruvian women. Presented at 10th World Congress on the Menopause, Berlin, Germany. 2002.
  29. Terzic MM, Dotlic J, Maricic S, Mihailovic T, Tomic-Race B. Influence of Red clover-derived isoflavones on serum lipid profile in postmenopausal women. *J Obstet Gynaecol Res*. 2009 Dec;35(6):1091-5.
  30. Campbell MJ, Woodside JV, Honour JW, Morton MS, Leathem AJ. Effect of red clover-derived isoflavone supplementation on insulin-like growth factor, lipid and antioxidant status in healthy female volunteers: a pilot study. *Eur J Clin Nutr*. 2004 Jan;58(1):173-9.
  31. Woods R. Effects of red clover isoflavones (Promensil) versus placebo on uterine endometrium, vaginal maturation index and the uterine artery in healthy postmenopausal women. *Journal of the British Menopause Society*. 2003;23.
  32. Atkinson C, Warren RML, Sala E, Dowsett M, Dunning AM, Healey CS, et al. Red clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial. *Breast Cancer Research*. 2004;6(3):170r-9r.
  33. Powles TJ, Howell A, Evans DG. Red clover isoflavones are safe and well tolerated in women with a family history of breast cancer. *Menopause Int*. 2008;14:6-12.