

COMPARISON OF EFFECT OF RED CLOVER & CONJUGATED ESTROGEN ON MENOPAUSAL SYMPTOMS AND THEIR IMPACT ON QUALITY OF LIFE IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Objective: The objective of the study was to evaluate and compare the effect of conjugated estrogen and red clover on menopausal symptoms and their impact on quality of life in postmenopausal women.

Methods: A prospective, open labeled, randomized, comparative, clinical study was conducted on 50 patients. The patients were randomly divided in two groups of 25 each to receive either of the following two treatments: group A: Tablet Red Clover 80 mg once in a day orally daily and group B: Conjugated Estrogen 0.625mg once daily orally for 12 weeks. Clinical assessment was carried out in all the patients over a period of 12 weeks. End points of efficacy were

Menopausal rating scale (MRS) for assessment of menopausal symptoms and their impact on Quality of life & Vaginal pH and maturation index to assess the effect of drugs on vaginal health. **Results:** In both the groups, there was statistically significant improvement in menopausal symptoms over a period of 12 weeks. There was more decrease in MRS with conjugated estrogen group as compared to red clover group but the difference was not statistically significant. The reduction in vaginal pH and improvement in vaginal maturation index were also more with conjugated estrogen but the difference was not statistically significant. **Conclusion:** Both the treatment i.e. red clover and conjugated estrogen were found to be efficacious in postmenopausal patients. On comparing the above mentioned treatment groups, the results of conjugated estrogen were comparable with red clover. Thus, red clover can also be used as an alternative to conjugated estrogen for the treatment of post menopausal symptoms.

KEYWORDS: Postmenopause, Menopausal Rating Scale, Red Clover; Conjugated Estrogen, Vaginal maturation index

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.^[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.^[4]

The general approach consists of concurrent adoption of both life style modifications and drug therapy in the management of menopause. Menopausal hormone therapy (MHT), in the form estrogen therapy or combined estrogen-progestin therapy, is the established treatment for postmenopausal women with moderate-to-severe menopausal symptoms.^[5] The recommendation 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.^[6,7] 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.^[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]

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, this study is done to evaluate and compare the efficacy of Red clover and Conjugated estrogen in postmenopausal women.

MATERIAL & METHODS

This was a prospective, open label, randomized, comparative clinical study conducted on 50 patients. An informed consent was obtained from all patients enrolled for the study. The study was approved by Institutional Review Board (IRB) and Institutional Ethics Committee (IEC). Study was in accordance with the principles of good clinical practice (ICH-GCP) and declaration of Helsinki.

An adequate number of patients were screened and selected as per the inclusion and exclusion criteria for the study. The eligible patients were randomly divided into two study groups with the help of computer generated random numbers i.e. Group A received Red Clover 80 mg once in a day orally and group B received Conjugated Estrogen 0.625mg once daily orally for a period of 12 weeks. Each study group had 25 patients who had completed the study as per protocol. During the study, patients were not permitted to take any non-study drugs.

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.

Clinical assessment was carried out in all the patients over a period of 12 weeks. End points of efficacy were Menopausal rating scale for assessment of menopausal symptoms and their impact on Quality of life & Vaginal pH and maturation index to assess the effect of drugs on vaginal health. Changes in vaginal pH and VMI were recorded at baseline and at the end of 6 and 12 weeks. MRS was assessed at baseline and then subsequently at the end of 2, 6, 9 and 12 weeks.

Menopausal rating scale (MRS)

MRS is designed to assess menopause specific health related quality of life and to measure the menopause-related complaints by rating a profile of symptoms. It is composed of 11 items, which are further divided into three subscales: (a) somatic - hot flushes, heart discomfort/palpitation, sleeping problems, muscle and joint problems; (b) psychological-depressive mood, irritability, anxiety, physical and mental exhaustion and (c) urogenital-sexual problems, bladder problems and dryness of the vagina. Each of the eleven symptoms are having a scoring scale ranging from “0” (no complaints) to “4” (very severe symptoms). The total score of the MRS ranges between 0 (asymptomatic) and 44 (highest degree of complaints).

Vaginal Maturation Index

The “Maturation Index” monitors the differentiation of the immature squamous cells towards their most evolved forms, parabasal, intermediate cells or the mature superficial cells, each representing a cellular layer. The index represents the relative number of each kind of cells per hundred cells counted. By maturation index, effect of estrogen on vaginal squamous cell layer can be assessed. The VMI is an indicator of the estrogenic effect on the vaginal wall, with a range of 0–49 indicating absent or low estrogenic effect, 50–64 moderate estrogenic effect, and 65–100 high estrogenic effect.

$$\text{Vaginal Maturation Index} = (\% \text{ Intermediate Cells} \times 0.5) + \% \text{ Superficial Cells}$$

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.

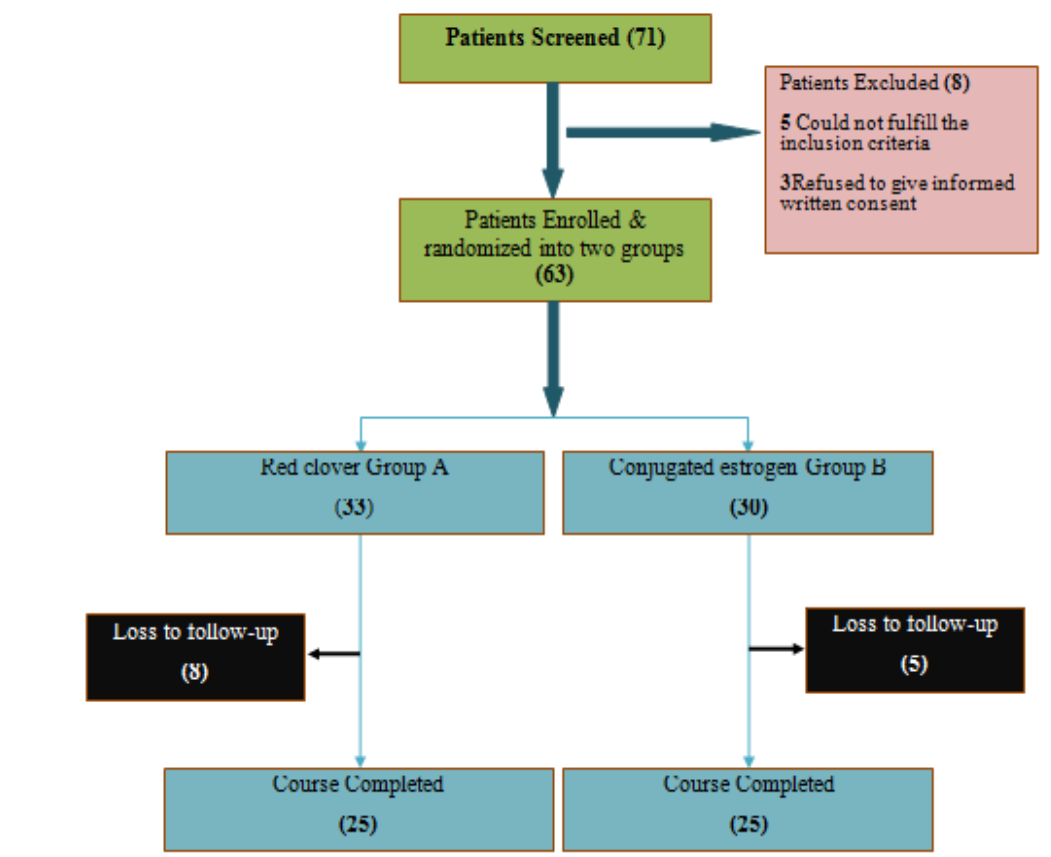
Data was expressed as Mean \pm SEM. Both intragroup and intergroup statistical analysis was done. Intragroup analysis for repeated measures was done using ANOVA for parametric data.

Intergroup analysis was done using unpaired 't' test for parametric data. A p-value <0.05 was considered as statistically significant.

RESULTS

Enrolment of patients for the study is shown in flowchart 1.

ENROLMENT OF STUDY POPULATION



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 ± 1.042 and $51.96 \pm .99$ (Mean \pm SEM) in group A and group B respectively.

Table.1: Comparison of Study Population Characteristics in both the Groups

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

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

The MRS 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) Table 2 shows the comparison of changes in somatic, psychomotor and urogenital scales in both the groups. Statistically significant reduction ($p < 0.05$) in all the parameters of menopause rating scale i.e. somatic, psychological and urogenital scale was observed over a period of 12 weeks in both the groups. Reduction in composite MRS in red clover group at 12 weeks was 20.5% whereas it was 23.6% in Conjugated Estrogens group. However, no statistically significant difference was observed between the two groups regarding the reduction of somatic, psychomotor, urogenital and composite MRS scales. The results were almost comparable. Figure 1 shows the percentage reduction in composite MRS in both the groups over a period of 12 weeks.

Table.2: Comparison of Changes in Somatic, Psychomotor and Urogenital Scales in Both the Groups

| | | Red Clover (Group A) | | Conjugated Estrogen (Group B) | | p value (inter-group) |
|------------------------|----------|--------------------------|-----------------------------|-----------------------------------|-----------------------------|--------------------------|
| | | Mean \pm SD | %reduction from baseline | Mean \pm SD | %reduction from baseline | |
| Somatic Scale | Baseline | 4.28 \pm 1.36 | - | 4.48 \pm 1.35 | - | 0.536 |
| | 2 weeks | 4.12 \pm 1.36 | 1.4 | 4.40 \pm 1.22 | 1.7 | 0.449 |
| | 6 weeks | 4.08 \pm 1.28 | 4.7 | 4.28 \pm 1.24* | 4.7 | 0.574 |
| | 9 weeks | 2.32 \pm .988* | 11.2 | 3.96 \pm 1.09* | 11.6 | 0.612 |
| | 12 weeks | 2.16 \pm .943* | 19.6 | 3.20 \pm .86* | 28.6 | 0.391 |
| Psychological scale | Baseline | 2.60 \pm .86 | - | 2.68 \pm .945 | - | 0.756 |
| | 2 weeks | 2.60 \pm .86 | 0 | 2.56 \pm 1.08 | 4.5 | 0.886 |
| | 6 weeks | 2.56 \pm .82 | 1.5 | 2.48 \pm 1.04 | 7.5 | 0.765 |
| | 9 weeks | 2.20 \pm .70* | 11.5 | 2.32 \pm .988* | 13.4 | 0.624 |
| | 12 weeks | 2.12 \pm .72* | 18.5 | 2.16 \pm .943* | 19.4 | 0.867 |
| Urogenital scale | Baseline | 1.80 \pm .64 | - | 2.00 \pm .81 | - | 0.341 |
| | 2 weeks | 1.76 \pm .59 | 2.2 | 1.80 \pm .76 | 10 | 0.837 |
| | 6 weeks | 1.76 \pm .72 | 2.2 | 1.78 \pm .78 | 11 | 0.576 |
| | 9 weeks | 1.52 \pm .58* | 15.5 | 1.64 \pm .63* | 18 | 0.492 |
| | 12 weeks | 1.44 \pm .50* | 20 | 1.52 \pm .58* | 24 | 0.608 |

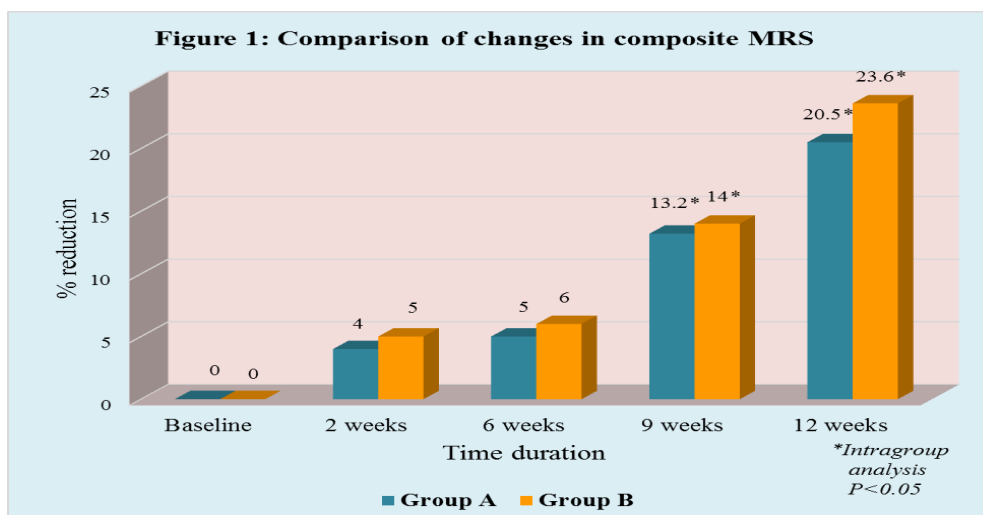
INTRAGROUP ANALYSIS:

**In group A- Comparison of values at end of week 9 and 12 with baseline values: showing statistical significance ($p < 0.05$).*

**In group B- Comparison of values at end of week 6, 9 and 12 with baseline values: showing statistical significance ($p < 0.05$).*

INTERGROUP ANALYSIS:

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



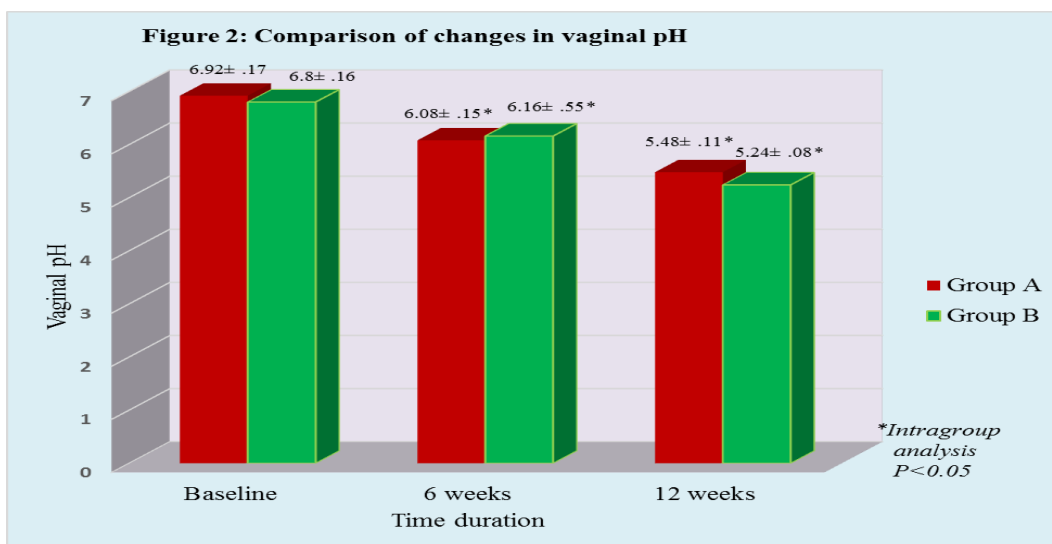
INTRAGROUP ANALYSIS

* Comparison of values at end of week 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 ($p > 0.05$)

There was statistically significant reduction in vaginal pH in both the groups over a period of 12 weeks (Fig – 2). In red clover group, the reduction was 1.44 whereas it was 1.56 in Conjugated Estrogens group at the end of 12 weeks. Moreover, it was observed that statistically significant improvement occurred in vaginal maturation index (Fig-3). In red clover group, mean improvement in VMI was 19.6 whereas it was 20 in Conjugated Estrogens group at the end of 12 weeks. However, no statistically significant difference was found between the two groups regarding the reduction of vaginal pH and improvement of VMI. The results were almost comparable. All the patients responded to the study medications, so escape treatment was not required in any of the patients of either group.



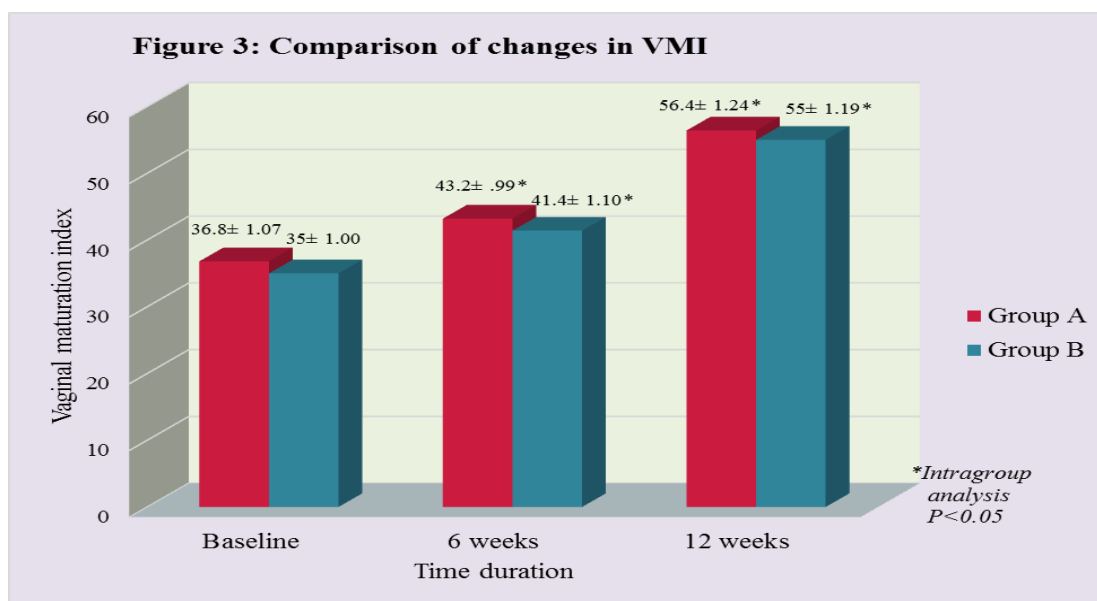
- All values are expressed as Mean ± SEM
- Group A: Red Clover 80 mg
- Group B: Conjugated Estrogen 0.625 mg

INTRAGROUP ANALYSIS:

* Comparison of values at end of week 6 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 ($p > 0.05$)



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

INTRAGROUP ANALYSIS:

** Comparison of values at end of week 6 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 ($p > 0.05$).

DISCUSSION

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. Treatment is indicated if menopausal symptoms interfere with a woman's daily functioning and quality of life. 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] Due to 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]

Analysis of the menopause rating scale in the present study showed that both the groups were comparable regarding the reduction of menopause rating scale and there was no statistically significant difference. Follow up visits showed an improvement in percentage reduction of mean menopause rating scale in both the groups. Reduction in MRS was seen as early as 2 weeks of treatment in red clover group (4%) as compared to their baseline values ($p < 0.05$) and this reduction was continuous over 12 weeks (20.5%). In the present study, fall in mean

MRS was also seen in the conjugated estrogen group in the week 2 (5%) which was continuous till week 12 i.e.23.6%.

In a triple blind placebo controlled study done by Shakeri et al, on 75 postmenopausal women, MRS at third month of study decreased from 20.41 to 9.86 in intervention group (Red clover) and 20.77 to 17.20 in placebo group.^[15]

In another study done by Ehsanpour et al, a total of 55 women completed the study, 28 subjects in red clover and 27 in placebo group. Mean score of total quality of life ($p < 0.001$ in both groups), mean score of quality of life in vasomotor domain ($p < 0.001$ in both groups), psycho-social domain ($p < 0.001$ in red clover and $p = 0.02$ in placebo group) and physical domain ($p < 0.001$ red clover and $p = 0.01$ placebo group) significantly reduced compared to the baseline values. However, the differences between two groups were significant neither for total quality of life nor for its domains.^[16]

The normal vaginal pH of a reproductive aged woman is 3.9 – 4.5. During premenopausal years, vaginal luminal pH ranges between 4.5 and 6, whereas lack of estrogen after the menopause is associated with alkalinization to about 6.5 – 7.

In our study, at the end of 6 and 12 weeks there was more reduction in vaginal pH with conjugated estrogen as compared to red clover but the difference was statistically not significant. In red clover group mean reduction in vaginal pH was from 6.92 (baseline) to 5.48 (12 week), hence the reduction was 1.44. In conjugated estrogen group mean reduction in vaginal pH was from 6.80 (baseline) to 5.24 (12 week), hence the reduction was 1.56.

In a study done by Marx et al, in a randomized, double-blind, multicenter clinical trial, 71 healthy postmenopausal women with vaginal atrophy (Vaginal Maturation Index ≤ 55) received either low-dose synthetic conjugated estrogens (SCE-A), 0.3 mg once daily or placebo for 16 weeks. Treatment with SCE-A for 16 weeks resulted in significant decrease in Vaginal pH from 6.2 at week -2 to 5.2 at week 16 with SCE-A compared to placebo ($P < 0.0001$).^[17]

Estrogen therapy decreases thickening and revascularizes the vaginal epithelium, increases the number of superficial cells (thereby increasing the Vaginal Maturation Index) Estrogen or combined hormone (estrogen-progestin) therapy is highly efficacious for managing the signs

and symptoms of urogenital atrophy. A low, effective estrogen dose may enhance patient acceptance and reduce side effects.

In present study, at the end of 6 and 12 weeks there was more improvement in VMI with red clover as compared to conjugated estrogen but the difference was statistically insignificant. In red clover group mean improvement in VMI was from 36.80 (baseline) to 56.40(12 week), showed an increase of 19.6. In red clover group mean improvement in VMI was from 35(baseline) to 55(12 week), showed an increase of 20.

In a study done by Marx et al, in a randomized, double-blind, multicenter clinical trial, 71 healthy postmenopausal women with vaginal atrophy (Vaginal Maturation Index ≤ 55) received either low-dose synthetic conjugated estrogens (SCE-A), 0.3 mg once daily or placebo for 16 weeks. Treatment with SCE-A for 16 weeks resulted in a highly significant ($P < 0.0001$) mean increase of 17.7 in the Vaginal Maturation Index compared to a mean increase of 4.1 with placebo treatment.^[17]

In another study, a meta-analysis reviewing randomized, placebo-controlled trials published between 1969 and 1995 determined that estrogen therapy, as compared to placebo, was efficacious in the treatment of post-menopausal women with signs and symptoms of vaginal atrophy.^[18]

In the Women's HOPE trial, which included a dose of 0.3 mg conjugated equine estrogens with and without medroxyprogesterone acetate at 1.5 mg /day, significant changes from baseline Vaginal Maturation Index were reported after 6 and 13 cycles respectively.^[19]

Woods et al, showed that the administration of red clover isoflavones, has also been correlated with a significant improvement in the vaginal maturation index.^[20]

Both the treatment groups i.e. red clover and conjugated estrogen were found to be safe and efficacious in postmenopausal patients (led to reduction in menopause rating scale, vaginal pH and improvement of vaginal maturation index).On comparing the above mentioned treatment groups, the results of conjugated estrogen were comparable with red clover. Though the sample size and study duration was small in this study, further research with larger groups and longer study period is required to support these findings.

CONCLUSION

Both the treatment groups i.e. red clover and conjugated estrogen were found to be efficacious in postmenopausal patients. On comparing the above mentioned treatment groups, the results of conjugated estrogen were comparable with red clover. Thus, red clover can also be used as an alternative to conjugated estrogen for the treatment of postmenopausal symptoms.

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