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Effect of bacosides enriched standardized extract of *Bacopa monniera* (BESEB-CDRI-08) on lipid profile and blood pressure of postmenopausal women: a pilot study

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Abstract
Postmenopausal women are more prone to the development of coronary heart diseases. Generally, elevated serum cholesterol and lipoprotein levels are used as primary biomarkers for cardiovascular diseases (CVD). The objective of this study was to evaluate the efficacy of bacosides enriched standardized extract of *Bacopa monniera* (BESEB-CDRI-08) on lipid profile and blood pressure of postmenopausal women. A single centered, randomized controlled, double blind trial was conducted on 25 postmenopausal women (mean±SEM; 52.00±2.67). The subjects were randomized and divided in 2 groups, group 1: treated with one capsule of BESEB-CDRI-08 (n=19) per day and group 2: treated with placebo capsule (n=6), containing 300 mg of lactose. The changes in the lipid profile (serum cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and VLDL), blood pressure and heart rate were evaluated. The results obtained after 4 months of treatment showed a significant decrease in serum triglycerides (p=0.002) and VLDL level (p=0.014) in BESEB-CDRI-08 treated group (as compared to baseline data). Serum cholesterol and LDL-cholesterol also decreased in BESEB-CDRI-08 treated group. Moreover, blood pressure (both systolic and diastolic) and heart rate also reduced in BESEB-CDRI-08 treated group ($p \leq 0.001$, p=0.002, p =0.001 respectively). However, the placebo treated group showed no significant change in lipid profile and blood pressure. These preliminary findings suggest that standardized extract of *B. monniera* (BESEB-CDRI-08) may significantly decrease the risk of CVD in postmenopausal woman.

Keywords: Cardiovascular diseases; *Bacopa monniera*; Menopause; Lipid profile.

1. Introduction
Menopause is a major life transition accompanied by profound changes on both emotional and physical levels. The menopausal transition occurs in a woman's reproductive life when the production of two primary hormones *viz*. estrogen and progesterone (released from the ovaries) vary dramatically and unpredictably [1]. These physiological alterations cause changes in glucose and insulin metabolism, body fat distribution, coagulation, fibrinolysis, vascular endothelial dysfunction and derangement of lipoprotein profile [2, 3]. Most of events as discussed above subsequently predispose an individual primarily towards various cardiovascular complications. Cardio vascular diseases (CVD) are the primary cause of death in women of western countries. The occurrence of CVD is distinct in men and women, as onset begins approximately 10 years later in women as compared to men [4]. Although women below the age of 50 years rarely develop CVD, however this incidence becomes equal in men and post menopausal women, suggesting that estrogen deficiency causes a rapid acceleration in CVD risk [5]. Moreover, premature and surgically induced menopause has also been found to increase the risk for CVD [6].
Currently, women are prescribed with different hormone replacement therapy (HRT) [7] to alleviate the symptoms associated with menopause. HRT basically contains one or more female hormones, commonly estrogen plus progestin [8]. However, Women's Health Initiative (WHI) under the National Institutes of Health in the USA suspended the components of HRT after concluding that they increased the risk of coronary heart disease, stroke, venous thromboembolism and invasive breast cancer. Because of these risks, new therapeutic approaches are being intensively investigated.
Ayurveda has an excellent solution for a safe and happy menopause. There are several *rasayanas* (rejuvenators) drugs that may be used to reduce menopausal complications [9]. *Bacopa monniera* is a common *rasayana* [10] that is used for enhancing memory, analgesia and epilepsy. The plant has been shown to possess a potent free radical scavenging, antioxidant [11],

cardio-protective [12], vasodilator [13], anti-inflammatory [14], calcium antagonistic [15], mast cell stabilizing [16], antiulcer [17] and anti addictive [18] properties.

One of our previous findings suggested that standardized extract of *B. monniera* (BESEB-CDRI-08, containing 55% of combined bacoside A and bacoside B) alleviated insomnia in postmenopausal women [19]. BESEB-CDRI-08 is an enriched preparation, chiefly containing steroidal saponins, bacosides A and bacosides B. It was reported that several saponins inhibit the intestinal absorption of cholesterol and reduce plasma cholesterol levels in a variety of experimental animal models [20, 21, 22, 23]. Recently, it was also reported that alcoholic extract of *B. monniera* may provide protection on various biochemical changes and aortic pathology in hypercholesterolemic rats [24]. Thus the plant may therefore be useful for therapeutic treatment of clinical conditions associated with hypercholesterolemia. Based on these findings it may be presumed that BESEB-CDRI-08 may have the cholesterol-lowering effect in postmenopausal women. However, no clinical data for the same has been reported yet. In this pilot study, we made an attempt to investigate the effect of BESEB-CDRI-08 on various primary clinical parameters *viz*. serum lipid profile, blood pressure and heart rate of postmenopausal women.

2. Subjects and methods

2.1 Subjects

The study was designed according to the declaration of Helsinki. Initially 30 postmenopausal women aged 50 and above with no menstrual periods for more than 12 consecutive months were included in this study. Subjects were excluded from participating in the study if they were suffering from kidney and liver dysfunction or were being treated with antidiabetic, antihypertensive, antihyperlipidemic, or hormonal replacement for menopause. Subjects who undergo major hospitalization or any surgery during past 3 years were also excluded from the study. All the protocols were approved by the Institutional Ethics Committee, Department of Pharmaceutical Sciences, Kumaun University and informed consent forms were duly signed by all the participants. Subjects were recruited in the study after a health screening. On the screening visit, women were responded to a questionnaire, which ascertained information about demographic characteristics. Women were also queried about menopausal symptoms covered by the menopause rating scale [25], gynecologic history, lifestyle, use of selected medications, physical activity, dietary and nutritional habits.

2.2 Clinical characteristics

Blood samples (post 12 h fasting) were obtained from all the subjects (after the questionnaire session) by vein puncture to measure hemoglobin and serum lipid profile enzymatically (ERBA diagnostic Mannheim GmbH). Height and weight were measured with subjects wearing light weight clothing and no shoes; body mass index (calculated as kg/m^2) was used as an estimate of obesity. Blood pressure was measured with a mercury sphygmomanometer after the subject had been seated quietly for at least 5 min. Women were also asked about the postmenopausal symptoms on the menopause rating scale and rated on a scale from 0 to 4 for none, mild, moderate, severe and very severe complaints [25]. Based on the above mentioned evaluation parameters, subjects were screened and randomized.

2.3 Study design and treatment

The study was a single-centre, 4 months, randomized, double-blind trial designed to investigate the effects of BESEB-CDRI-08 on post menopausal women. BESEB-CDRI-08 capsule were obtained from the Lumen Marketing Company, Chennai, the sole licensee of manufacturing and marketing of Central Drug Research Institute (CDRI) patented process of *B. monniera* extract. Based on the screening results (compliance with the subject inclusion criteria), twenty five healthy female postmenopausal volunteers aged between 50-60 years (mean±SEM; 52.00±2.67) and weighing between 55 and 85 kg (mean±SEM; 63.60±2.60) were randomly assigned to one of the two treatment groups: B. monniera treated group *(*BESEB-CDRI-08 administered as one capsule per day, each capsule containing 300 mg of the dried extract); Placebo treated group (One capsule containing 300 mg of lactose).

Both the capsules were identical in shape, color, smell, taste and weight. The dose of *B. monniera* was based on the standard clinical dose recommended by CDRI, Lucknow. The protocol required five visits, one screening visit and four treatment visits.

2.4 Procedure

After screening and randomizing, fixed numbers of capsules were given to the eligible volunteers into a dark colored coded container. In addition to the trial regime (i.e. 30 capsules for 4 weeks), additional capsules ranging in number from 1-10 (randomly allocated) were also placed in the bottles so that compliance could be accurately examined. After the completion of 4 weeks, participants were asked to bring their bottles and the remaining capsules were counted. Participants were excluded if greater than 10% of the total number of capsules required were not consumed by the end of the 4 weeks. At each study visit including the initial visit all volunteers were gone through the same protocols. A clinical assessment of each subject was carried out at the end of each month up to 4 months. Subjects were received weekly phone calls over the treatment period to monitor any adverse effects and to enhance compliance.

2.5 Serum lipid profile assay

Blood samples were withdrawn from each participant after 12 hours of fasting. Samples were centrifuged, and serum was stored at −70 ºC until analysis. Triglycerides, total cholesterol and HDL-cholesterol were assayed enzymatically using commercially available kits (ERBA diagnostic Mannheim GmbH). LDL-cholesterol was calculated according to the Friedewald's equation. VLDL was calculated as 1/5 of triglycerides. All assays were performed according to the manufacturers' instructions.

2.6 Statistical considerations

All the raw data obtained was analyzed using Sigma Stat Software (Jandel Scientific Sigma Stat 2.0). Groups' baseline data are presented in Table 1 as mean±SEM. These values were analyzed by Student's *t*-test for independent samples to verify if there was any difference between groups at study baseline.

The values obtained during study for each parameter at different time point was statistically analyzed by using one way analysis of variance (ANOVA) followed by Tukey's test. Results were considered significant at 95% confidence interval with $p \leq 0.05$.

3. Results

A total of 25 patients completed the 4 months study; 19 for BESEB-CDRI-08 and 6 for placebo group. Only subjects who completed 4 months of treatment and took over 90% of their expected number of drugs were included in the efficacy analysis. A few adverse effects were observed like skin dryness, itching and abdominal upset that did not last for more than 2 months. Baseline characteristics of subjects are shown in Table 1. No significant difference was noticed among the two treatment groups at baseline examination for any parameter.

Table 1: Baseline characteristics of randomized subjects in two treatment groups.

Parameters	BESEB-CDRI-08 (*n=19*)	Placebo (*n=6*)
Age (in years)	55±1.025	57±1.983
Weight (in kg)	63±2.201	61±1.072
BMI (kg/m^2)	28±1.105	27±1.211
Age at menopause(years)	49±0.952	50±0.994
Time since last menstrual period(years)	6.1±0.879	5.8±1.223
Hemoglobin(g/dL)	11.774±1.247	12.633±2.299
Total cholesterol(mg/dL)	190.316±9.024	188.83±18.676
LDL Cholesterol(mg/dL)	114.895±7.872	128.833±14.337
Serum Triglycerides(mg/dL)	184.105±10.983	164±21.323
HDL cholesterol(mg/dL)	47.053±1.953	46.167±3.953
VLDL(mg/dL)	35.632±3.48	31.8±2.725
Systolic Blood Pressure(mmHg)	140.158±3.373	139.833±5.648
Diastolic Blood Pressure(mmHg)	83.158±1.678	82.333±4.507
Pulse rate(per min)	79.158±1.617	75.833±1.222

Data expressed as mean±SEM. BMI: Body mass index; LDL: Low density Lipoprotein; HDL:High density lipoprotein; VLDL: Very low density lipoprotein.

Change in various parameters after the completion of study in both BESEB-CDRI-08 and placebo treated group are shown in Table 2. There was no significant difference observed in serum total cholesterol, LDL cholesterol and HDL cholesterol after treatment in both BESEB-CDRI-08 and placebo treated group. However, there was a significant decrease in serum triglyceride (p=0.002) and VLDL level (p=0.014) in BESEB-CDRI-08 treated group, when compared with baseline data (Fig.1)

Table 2: Lipid profile, blood pressure and heart rate of each group at baseline and after completion of study.

Parameters	BESEB-CDRI-08 (*n=19*)		Placebo (*n=6*)	
	Basal	4th month	Basal	4th month
Total cholesterol (mg/dL)	190.316±9.024	183.684±3.775	188.83±18.676	203±12.872
LDL Cholesterol (mg/dL)	114.895±7.872	108.505±6.773	128.833±14.337	135.533±11.844
Serum Triglycerides (mg/dL)	184.105±10.983	131.60±5.923*	164±21.323	162.333±9.687
HDL cholesterol (mg/dL)	47.053±1.953	47.00±1.025	46.167±3.953	44.5±1.147
VLDL (mg/dL)	35.632±3.48	24.32±1.01*	31.8±2.725	36.467±1.937
Systolic Blood Pressure (mmHg)	140.158±3.373	124±1.562**	139.833±5.648	127.667±2.642
Diastolic Blood Pressure (mmHg)	83.158±1.678	75.588±1.627*	82.333±4.507	76±1.789
Heart rate (per min.)	79.158±1.617	71.176±1.089**	75.833±1.222	72.5±1.335

Data expressed as mean±standard error. *p≤0.05 and **p≤0.001 as compared to baseline.

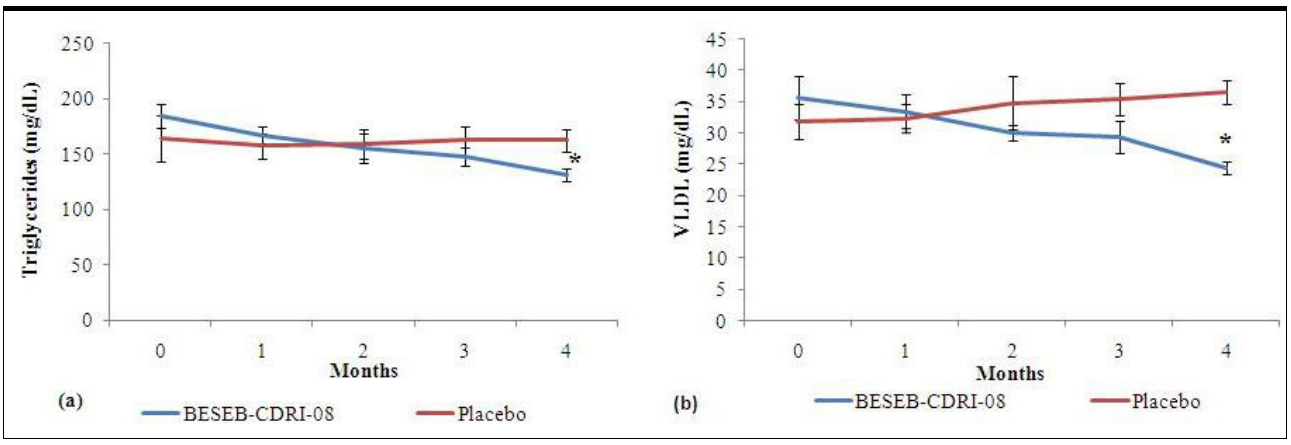

Fig 1: Effect of treatments on serum triglyceride and VLDL level of postmenopausal women. Data expressed as mean±SEM (n=19, in BESEB-CDRI-08 treated group and n=6, in placebo treated group). * p≤0.05 as determined with one way ANOVA followed by Tukey's test when comparing to the baseline data.

Systolic (p<0.001) and diastolic blood pressure (p=0.002) were found to be decreased in BESEB-CDRI-08 treated group, whereas no such change was observed in placebo treated group (Fig.2). Also heart rate (p =0.001) was significantly reduced in BESEB-CDRI-08 treated group (Fig. 3).

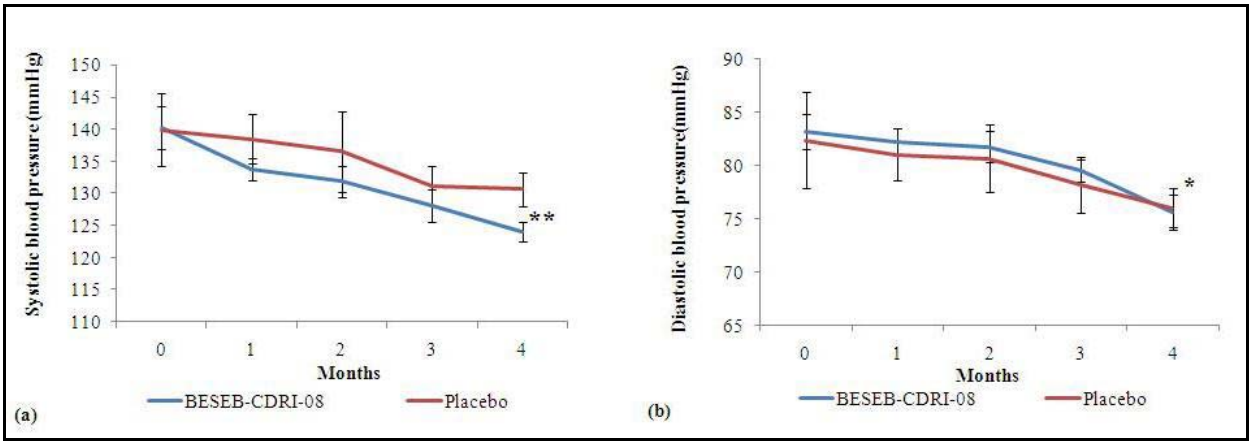

Fig 2: Effect of treatments on systolic and diastolic blood pressure of postmenopausal women. Data expressed as mean±SEM (n=19, in BESEB-CDRI-08 treated group and n=6, in placebo treated group). * p≤0.05 and ** p≤0.001 as determined with one way ANOVA followed by Tukey's test when comparing to the baseline data.

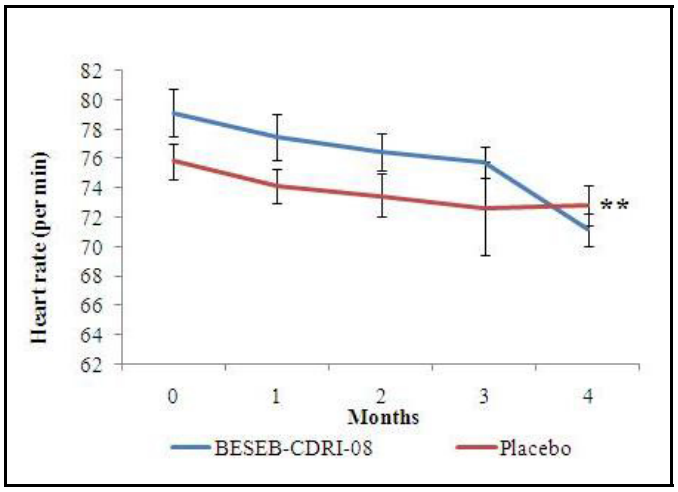

Fig 3: Effect of treatments on heart rate of postmenopausal women. Data expressed as mean±SEM (n=19, in BESEB-CDRI-08 treated group and n=6, in placebo treated group). ** p≤0.001 as determined with one way ANOVA followed by Tukey's test when comparing to the baseline data.

4. Discussion

The menopausal transition is frequently associated with CVD, hot flushes, night sweats, painful joints, poor self-rated health and sleeping problems. The management of vasomotor and other menopausal symptoms is complex, and the treatment remains contentious. It is noteworthy that most women can tolerate menopausal symptoms, if the symptoms are mild and transient [26].

The present study showed that BESEB-CDRI-08 significantly reduced both triglyceride and VLDL level in postmenopausal women. Significant reduction was also observed in blood pressure (both systolic and diastolic) and heart rate after 4 months of treatment with BESEB-CDRI-08. These findings may support its use in reducing the risk of CVD in postmenopausal women. However, its effect on total cholesterol, LDL and HDL level was showing some improvement but it was not significant as compared to baseline. This may be due to the fact that herbal preparations require longer duration of treatment for showing its complete effects. This was also seen in one report where positive effects on learning and memory were observed following 12 weeks administration [27]. Another study showed memory enhancing effects following 4 weeks, however this study was confounded by concomitant improvements in anxiety and the use of a higher dose of *B. monniera* [28].

The effect of standardized extract of *B. monniera* have been established in various other diseases by clinical trials as discussed earlier, but its effect on postmenopausal symptoms remain untouched. This is the first report of a randomized control, double blind study, on postmenopausal women using standardized extract of *B. monniera* to assess its effectiveness on CVD.

This study implicated that daily administration of standardized extract of *B. monniera* (BESEB-CDRI-08) in postmenopausal women was effective in controlling the physiological parameters that predisposes an individual to CVD. This herbal preparation was found to be well tolerable and safer as compared to other available treatments as the associated adverse effects were very less and easily tolerable. Therefore it can be used as a more effective and less toxic alternative of HRT. Moreover, HRT is also associated with many complications which have been reported in various clinical studies. One such finding implicates that conjugated estrogens plus medroxyprogesterone acetate reduced insulin sensitivity in menopausal women without affecting body composition or body fat distribution. The reduction in insulin sensitivity was reversible after discontinuing HRT [29]. These results gave strength to the use of herbal drugs in postmenopausal women and open a new venue to treat the metabolic disorders in menopausal women.

5. Conclusion

Based on these findings, it was reported that daily administration of standardized extract of *B. monniera* (BESEB-CDRI-08) may reduced the risk of CVD in menopausal women. However, the findings reported are preliminary and thus needs further mechanistic studies to understand the exact mode of action of this preparation at cellular and molecular level.

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