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A Novel Extract of Fenugreek Husk (FenuSMART™) Alleviates Postmenopausal Symptoms and Helps to Establish the Hormonal Balance: A Randomized, Double-Blind, Placebo-Controlled Study

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Despite the widespread use of hormone replacement therapy, various reports on its side effects have generated an increasing interest in the development of safe natural agents for the management of postmenopausal discomforts. The present randomized, double-blinded, placebo-controlled study investigated the effect of 90-day supplementation of a standardized extract of fenugreek (*Trigonella foenum-graecum*) (FenuSMART™), at a dose of 1000 mg/day, on plasma estrogens and postmenopausal discomforts. Eighty-eight women having moderate to severe postmenopausal discomforts and poor quality of life (as evidenced from the scores of Greene Climacteric Scale, short form SF-36® and structured medical interview) were randomized either to extract-treated ($n = 44$) or placebo ($n = 44$) groups. There was a significant ($p < 0.01$) increase in plasma estradiol (120%) and improvements on various postmenopausal discomforts and quality of life of the participants in the extract-treated group, as compared with the baseline and placebo. While 32% of the subjects in the extract group reported no hot flashes after supplementation, the others had a reduction to one to two times per day from the baseline stages of three to five times a day. Further analysis of haematological and biochemical parameters revealed the safety of the extract and its plausible role in the management of lipid profile among menopausal women. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: Trigonella foenum-graecum; fenugreek; menopausal symptoms; phytoestrogens; Greene Climacteric Scale; estradiol; quality of life.

INTRODUCTION

Menopause, the natural end of a female reproductive life, is usually defined as the permanent cessation of menstruation resulting from a complex phenomenon involving many endocrinological changes followed by the onset of the loss of ovarian follicular activity (Burger *et al*., 2007). The period of menstrual cycle irregularity preceding the menopause is generally regarded as the menopausal transition stage, which may even begin at 35 years of age with a progressive decline in fecundity and peaks during the second half of the fourth decade of life and finally the culmination around 51 years (Santoro, 2005; Burger *et al*., 2007). Menopausal transition has been shown to be associated with marked hormonal instability contributed by the imbalance in the hypothalamo–pituitary–ovarian axis (Santoro, 2005; Burger *et al*., 2007). Melbourne Women's Midlife Health Project study has provided significant insight into the endocrinology of menopause as primarily characterized by concomitant decrease in the levels of inhibin and anti-Mullerian hormones (Burger *et al*., 2007; Freeman *et al*., 2012). The decline in inhibin was shown to induce an increase in the levels of follicular-stimulating hormone with a significant decrease in estradiol and androgen levels (Freeman *et al*., 2012). While the decrease in androgens is responsible for low libido and reduced frequency of sexual activity, deprivation of estradiol levels induces a variety of discomforts leading to the poor quality of life (Yasui *et al*., 2012). The most prevailing postmenopausal symptoms were categorized as clusters of psychological (palpitations, nervousness, anxiety, depression, insomnia, mood changes, irritability, skin dryness), physical (dizziness, headaches, breathing problems, joint/muscle pain, numbness, vaginal changes), vasomotor (hot flashes, night sweats, sexual dysfunction, bleeding problems) and sexual (decreased libido, urogenital problems) complaints along with osteoporosis (Burger *et al*., 2007; Cray *et al*., 2012; Rozenberg *et al*., 2013). Thus, menopause involves various facets, namely physiological, psychological and sociocultural issues that are intertwined.

Because hypoestrogenism was identified as the main reason for menopausal discomforts, estrogen

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administrations along with a loving care of the life partner was widely suggested to alleviate the systemic symptoms of postmenopause and genital atrophy (Cray *et al*., 2012; Rozenberg *et al*., 2013). Hormone replacement therapy (HRT) either with estrogen or with a combination of progesterone and applications of genital topical preparations has been mainly practicing for the management of menopausal symptoms (Rozenberg *et al*., 2013). However, various reports on its serious side effects such as enhanced probability of breast cancer, stroke and cardiovascular diseases have raised a serious concern over the use of HRT (Rozenberg *et al*., 2013).

It was widely studied that certain plant-derived natural compounds having estrogenic effects (phytoestrogens) could be used for the management of menopausal symptoms owing to their selective estrogen receptor modulating (SERM) properties (Pitkin, 2012). Soy isoflavones, Korean red ginseng (*Panax ginseng*) and red clover (*Trifolium pretense*) were reported to be effective in managing the climacteric complaints (Geller *et al*., 2009; Izzo *et al*., 2016). Black cohosh (*Actaea racemose*) is yet another botanical agent that was earlier considered as a phyto-SERM although the recent understanding is that its efficacy mediates through actein-like triterpene compounds and serotonin analogues possessing GABA-ergic activity (Wuttke and Seidlová-Wuttke, 2015). However, these herbal agents have many shortcomings including the non-compelling evidences of efficacy, genetic modifications and side effects (Geller *et al*., 2009; Izzo *et al*., 2016). Thus, there exists a tremendous interest for both novel phyto-SERMs (natural hormonal agents) and non-estrogenic compounds that are derived from safe food components capable of ameliorating the menopausal issues (Pitkin, 2012).

In the present paper, we investigated the safety and effectiveness of an extract of fenugreek husk (*Trigonella foenum-graecum*) rich in phytoestrogenic compounds (*FenuSMART*™; *hereinafter referred to as 'FHE'*) for the management of postmenopausal discomforts. Fenugreek, a kitchen spice generally recognized as safe (GRAS) by US Food and Drug Administration, is a popular medicinal herb possessing a wide range of applications in various traditional systems of medicine (Nathiya *et al*., 2014; Yadav and Baquer, 2014). Modern scientific research has identified it as a rich source of steroidal saponins, an alkaloid 'trigonellin' and a non-proteinogenic amino acid '4-hydroxyisoleucine' having the ability to bind to the estrogen receptors to mediate estrogenic and androgenic effects (Sreeja and Anju, 2010; Steels *et al*., 2011; Nathiya *et al*., 2014). Fenugreek seeds were clinically evaluated for premenopausal and postmenopausal discomforts (Hakimi *et al*., 2005; Hakimi *et al*., 2006; Abedinzade *et al*., 2015). Rao *et al.* (2015) reported the ability of a hydroethanolic extract of fenugreek seeds in improving the sexual function of healthy menstruating women. It was also reported that fenugreek seed extract has a potential neuroprotective action in patients with Parkinson's disease and demonstrated the safety and beneficial effect when used as an adjuvant to L-Dopa therapy (Nathan *et al*., 2014). However, no information was available on a standardized fenugreek extract and its effect on the management of postmenopausal discomforts.

MATERIALS AND METHODS

Study material. A proprietary hydroethanolic extract of fenugreek seed husks (FHE) rich in protodioscin, trigonellin and 4-hydroxyiosleucine (patent pending and registered formulation as 'FenuSMART™') was obtained from M/S Akay Flavours & Aromatics Pvt., Ltd., Cochin, India. FHE was having drug extract ratio of 18:1 (w/w) with respect to fenugreek husks (1 kg FHE was equivalent to 24 kg of dried fenugreek seeds) and characterized by the HPLC finger print profile as shown in Fig. 1. Food grade microcrystalline cellulose was employed as the placebo. Both FHE and placebo were analysed for the quality requirements as per US Pharmacopeia (USP) <561> requirements related to microbial content, aflatoxins, heavy metals and pesticides (USP, 2014). Hard-shell, two-piece gelatin capsules, each containing 250 mg of fenugreek husk extract (FHE) or placebo, was used for the study. Safety of FHE was assessed by lethal dose (LD_{50}), acute (14 days) and repeated dose subchronic (90 days) toxicity studies (data not given).

Study design and subjects. The present study was designed and conducted in a randomized, double-blinded, placebo-controlled manner. The study was carried out at M/S Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bangalore, India, under the supervision of a qualified gynaecologist and nutritionist. The study subjects were elected from symptomatic menopausal women, who attended to the gynaecologist for medical consultancy and treatment. The study was in accordance with the clinical research guidelines of Government of India following the protocol evaluated and approved by the institutional ethical committee at M/S Sri Jayadeva Institute of Cardiovascular Science and Research, Bangalore, India (Reg. No. ECR/423/Inst/KA/2013). The natural process of menopause, hormonal change, postmenopausal discomforts, its management methods and the objective of the present study were detailed to the subjects. A written consent was also obtained from all individuals who were willing to take part in the study.

A total of 130 natural menopausal women subjects, aged between 45 and 58 years, experiencing postme-

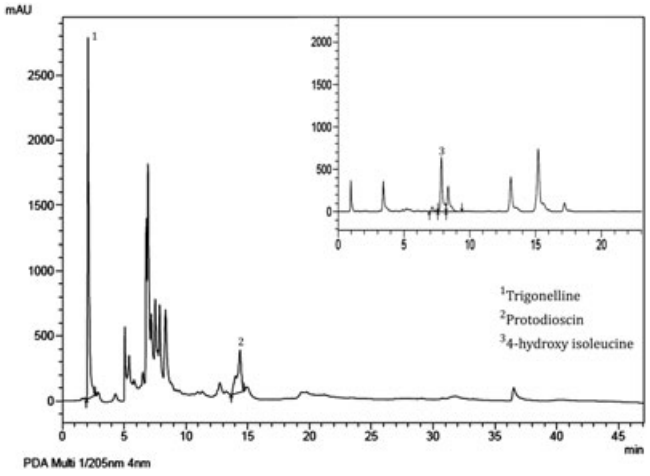

Figure 1. HPLC profile of fenugreek husk extract (FHE) used in the present study. Trigonelline (peak 1) and protodioscin (peak 2) were detected in a single HPLC run. 4-Hydroxyisoleucine was monitored in a separate run and given in inset as peak 3.

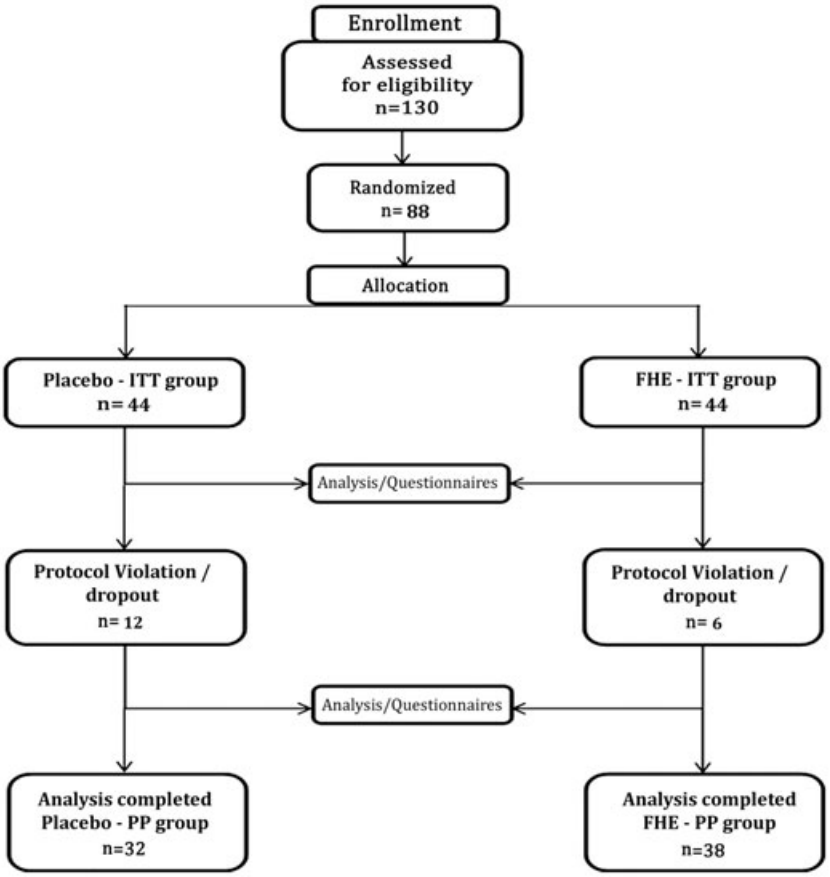

Figure 2. Schematic representation of the subject participation in trial.

nopausal discomforts were selected by purposive sampling. The primary inclusion criteria included those who have not experienced menses for at least 12 months and had their last menses within the past 3 years. Occurrence of moderate to severe postmenopausal discomforts with Green Climacteric Scale (GCS) mean score of ≥25 and a minimum of three hot flashes/day during the last 3 to 5 weeks were the decisive criteria. Prior use of HRT, family history of breast cancer, personal history of malignant neoplasm and hospitalization during the last 3-month period were considered for exclusion. Subjects having the prevalence of any cardiac risk factors and those under any medication or dietary supplementation were also excluded from the study. Besides the inclusion/exclusion criteria, baseline socioeconomic variables (age, race, civil status, marital status, education and age of menopause) were also noted. Out of 130 subjects, 42 women refused to participate in the study. Eighty-eight women were randomized to two intervention arms to receive FHE ($n=44$) or placebo ($n=44$). Sequentially numbered airtight identical containers containing 120 numbers of 250 mg capsules of either FHE or placebo were provided on visit 1 (0 days) and visit 2 (after 45 days). The participants were instructed to take two capsules per day [(250 mg × 1) after breakfast and (250 mg × 1) after dinner] for 1 week to adjust with the intake and later advised to take four capsules per day [(250 mg × 2) after breakfast and (250 mg × 2) after dinner] for another 12 weeks. The degree of adherence of the subjects was assessed by 'count pills' strategy. The efficacy of blinding was assessed by asking the participants and the investigator to guess to which group the patient was assigned. A consort flow diagram describing the design and subject participation in the study is shown in Fig. 2. The subjects were monitored on a weekly basis through regular telephonic follow-ups and short message services, through which the daily drug administrations and the details of side effects or discomforts (if any) were enquired. Details of the analyses performed on each of the visits during the study period were as shown in the succeeding texts.

Visit 1 (baseline/0th day)	Weekly	Visit 2 (45th day)	Weekly	Visit 3 (90th day)
Demographic data	SMS/telephone follow-ups		SMS/telephone follow-ups	
Anthropometric data		Blood pressure		Anthropometric data
Serum estradiol and calcium levels		Adverse events?		Serum estradiol and calcium levels
Blood pressure				Blood pressure
Blood tests (Hb, RBS, lipid profile)		Questionnaires (GCS and SF-36)		Blood tests (Hb, RBS, lipid profile)
Questionnaires (GCS and SF-36)				Questionnaires (GCS and SF-36)

Sample size and randomization. The sample size was presumed to be 90 from the statistical significance level of 0.05, power of test of 80% with an estimated standard deviation and difference of GCS between groups at the baseline. Although the initial attempt was to have a total of 100 subjects, the difficulty in the availability of interested subjects satisfying eligibility criteria restricted the number of subjects to 88 for randomization. The participants were randomly allocated by permuted-block randomization (block size = 4), employing the computer-generated allocation table (www.randomization.com).

Quality of life evaluation. Short Form-36 (SF-36®; The Health Institute, Boston, MA, USA) Health Survey was used to evaluate the quality of life of the participants during the course of the study. SF-36® was composed of 36 questions to allow the measurement of eight aspects of the quality of life: general, physical and mental health state, physical and social functioning, physical and emotional health, pain and vitality (Ware and Sherbourne, 1992). Data were collected at baseline (visit 1; 0 day) prior to the treatment, after 45 days (visit 2) and also at the end of intervention (visit 3; 90 days).

The Greene Climacteric Scale. The degree with which a participant was bothered by the postmenopausal symptoms was evaluated by the GCS, a comprehensive and validated tool consisting of 21 questions (Greene, 1998). Eleven statements in the GCS pertained to psychiatric symptoms (mainly anxiety and depression), seven statements assessed physical aspects, two statements to monitor vasomotor symptoms and the final one for the disorder in libido. The severity of the symptoms was scored as 0 (no symptoms), 1 (mild), 2 (moderate) and 3 (severe). Data were collected at baseline (visit 1; 0 day), after 45 days (visit 2) and also at the end of intervention (visit 3; 90 days). Questionnaires were completed in face-to-face interviews by the participants during their visits.

Biochemical/haematological analysis. Blood was collected by vein puncture and taken into EDTA/non-EDTA vials for assaying haematological and biochemical parameters. Serum was separated by centrifugation at 6000 rpm at 4 °C for 10 min and stored at −80 °C for further analysis. The analyses were performed at baseline and at the end of the study period. Haemoglobin (Hb) content was determined using automated cell counter (Model-Diatron, Wein, Austria). Random blood sugar levels were checked using ACCU-CHEK Active glucose test strips and an ACCU-CHEK Active glucose meter (Roche Diagnostic GmbH, Mannheim, Germany). Serum calcium levels were estimated by *O*-cresolphthalein method (Stern and Lewis, 1957), and the serum estradiol levels were measured using automated electrochemiluminescence immunoassay (Lee *et al.*, 2006). The serum lipid parameters such as total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were estimated by standard laboratory protocols. The very-low-density lipoprotein (VLDL) cholesterol was estimated by the Friedewald equation (VLDL = triglyceride / 5) and LDL cholesterol by the equation LDL = total cholesterol − (HDL + VLDL).

Anthropometric measurements. Anthropometric data comprising body mass index (BMI), mid-arm circumference, waist circumference, hip circumference and waist/hip ratio were measured as per standard guidelines (de Onis and Habicht, 1996). All anthropometric measures were taken under fasting conditions, and participants were wearing lightweight clothing and no shoes. The BMI (BMI = weight/height^2) was used to assess weight variation. Body weight was evaluated using a digital scale, and the height was measured in standing position using a tape meter graduated to the nearest 0.5 cm. Waist circumference was measured to the nearest 0.5 cm midway between the lowest rib margin and the top of iliac crest, and the hip circumference was measured at the largest posterior extension of the buttocks using an inelastic tape. Measurements were taken at the end of a normal respiration, while subjects stood erect with arms hanging loosely at sides and feet were together. The waist/hip ratio was used to assess body fat distribution by considering <0.8 as a gynaecoid pattern and ≥ 0.8 as an android pattern.

Statistical analyses. The primary evaluation of the efficacy data included the SF-36 and GCS data obtained at the baseline and after 90 days of treatment. The values are expressed as mean ± SD. The statistical significance was compared between untreated and treated by one-way analysis of variance followed by an appropriate *post hoc* test (Tukey's multiple comparison test) using Graphpad InStat software (version 3.05). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Study protocol and participant demographics

The typical protocol of the present study involving volunteer screening, enrolment and execution was given in Fig. 2. Out of the 130 postmenopausal subjects screened, 42 individuals were neither selected nor willing to participate in the study. Among the allocated subjects, there was a 27.3% dropout from placebo and 13.6% dropout from the treatment group, mainly because of their difficulty to appear for follow-ups and strictly adhere to the conditions of the treatment regime. None of the dropout was because of any adverse effects or intolerance of the fenugreek extract (FHE). Participants in both placebo and treatment groups were similar in terms of the personal and societal characteristics (Table 1). The average age of the participants in the placebo group was 52.97 ± 4.57 years and that of the treatment group was 53.31 ± 4.32 years respectively. Most of the participants in both the groups had high school education or above and were married house wives. The participants (73%) in the treatment group and 78% in the placebo group had no habit of any kind of exercise, other than the day-to-day housekeeping. The participants in both the treatment and placebo groups had

Table 1. Demographic characteristics of the study participants in the placebo and FHE-treated groups

Variables	Placebo (n = 32) mean ± SD	FHE (n = 38) mean ± SD
Age	52.97 ± 4.57	53.31 ± 4.32[a]
Body mass index (kg/m^2)	26.88 ± 4.99	26.95 ± 2.99[a]
Education	n (%)	n (%)
Below high school	12 (37.5)	17 (44.74)
High school and above	20 (62.5)	21 (55.26)
Occupation		
Employed	8 (25)	9 (23.68)
Home-maker	24 (75)	29 (76.32)
Marital status		
Married	30 (93.75)	33 (86.84)
Widowed	2 (6.25)	4 (10.53)
Divorced	0 (0)	1 (2.63)
Residence		
Urban	13 (40.63)	22 (57.89)
Rural	19 (59.38)	16 (42.11)
Exercise		
Never	25 (78.13)	28 (73.68)
Often	2 (6.25)	2 (5.26)
Daily	5 (15.63)	8 (21.05)

[a]$p > 0.05$, 'non-significant', when FHE compared with placebo.

their last menstrual period before 12 months. Biochemical analysis indicated no significant differences on serum hormone levels and metabolic profiles among the subjects in both the treatment and placebo groups at the baseline. The compliance rate of the test material was >95% for both placebo and FHE groups.

Effect of fenugreek husk extract on climacteric symptoms and quality of life

At baseline, the mean ± SD of the GCS total score was 34.83 ± 6.87 for the FHE group and 34.25 ± 7.45 for the placebo group, with no significant difference. The groups also exhibited approximately the same scores for all GCS subscales in the baseline (Table 2). Further treatment provided a significant reduction in the GCS score of FHE group: 24.82 ± 5.42 by the end of 45 days and 19.64 ± 4.28 after 90 days. The reduction in the placebo group was observed as 32.52 ± 8.24 after 45 days and then to 30.49 ± 5.23 by the end of study period. The observed decrease in total GCS score was significantly lower [95% confidence interval: −15.19 (−17.81 to −12.57), $p < 0.001$] than the placebo group [95% confidence interval: −3.71 (−6.94 to −0.49), $p > 0.05$]. As shown in Table 2, each of the constituting subscales corresponding to the symptoms of psychological, vasomotor, physical and sexual disorders was also significantly lower in the FHE group when compared with the values of the placebo group ($p < 0.001$). The anxiety scale showed a significant improvement ($p < 0.001$) in the FHE group, as compared with the baseline (58.9%) and placebo (53.9%). There observed a significant reduction ($p < 0.001$) in the average score corresponding to the depression in the FHE group, as compared with the baseline (47.7%) and placebo group (50%). GCS scores corresponding to physical and vasomotor symptoms were also significantly decreased (47.7 and 49.2% respectively; $p < 0.001$) as compared with the baseline (Table 2). Hot flashes showed 47.8% ($p < 0.001$) decrease with a reduction of average one to two times per day from the baseline stages of three to five times a day. Approximately 32% of the subjects reported no hot flashes after 90 days of supplementation. The mean score of vaginal dryness and irritability decreased in the study group from 42.1 and 65.8% to 18.4 and 15.8% respectively ($p < 0.001$), as compared with the reduction in the placebo group (from 51.3 and 58.8% to 50.3 and 56.2% respectively) (Fig. 3). Likewise, there was a significant reduction ($p < 0.001$) in other symptoms such as night sweats (57.1%), mood swings (68.2%), insomnia (75%) and headaches (53.9%) among the FHE-treated group when compared with the placebo group (Fig. 3).

A breakdown of mean SF-36 scores for different menopausal symptoms categorized across the eight health-related domains and the total physical and the mental component scores is presented as a spydergram (Fig. 4). The data demonstrated a significant improvement (73% of subjects) in the quality of life of the participants in the FHE group, as evident from the reduced physical/mental fatigue ($p < 0.001$) and enhanced interest on daily works with stability of mind and concentration, as compared with the placebo (32.5% of subjects). The participants in the FHE group reported a significant increase in their overall health functioning, particularly with regards to general well-being (11.9% increase, $p < 0.05$) and mental health (9% increase,

Table 2. Comparisons of the FHE supplemented and placebo groups in terms of the Greene Climacteric Scale (GCS) total and individual scores at baseline and after 90 days

	Placebo (n = 32)		FHE (n = 38)	
Symptoms	Baseline	Final	Baseline	Final
Psychological	17.85 ± 5.39	15.29 ± 3.98[a]	17.94 ± 5.15[a]	10.21 ± 2.76[b,b]
Anxiety	1.71 ± 0.94	1.65 ± 0.88[a]	1.85 ± 1.02[a]	0.76 ± 0.78[b,b]
Depression	1.76 ± 0.89	1.82 ± 0.87[a]	1.74 ± 0.79[a]	0.91 ± 0.62[b,b]
Physical	9.74 ± 2.68	8.21 ± 2.73[a]	9.85 ± 2.82[a]	5.15 ± 1.42[b,b]
Vasomotor	4.32 ± 1.43	4.28 ± 1.41[a]	4.35 ± 1.54[a]	2.21 ± 1.37[b,b]
Loss of sexual interest	1.85 ± 1.05	1.82 ± 0.99[a]	1.88 ± 1.15[a]	1.53 ± 1.02[b,b]
Total score	34.25 ± 7.45	30.49 ± 5.23[a]	34.83 ± 6.87[a]	19.64 ± 4.28[b,b]

Data are given as mean ± SD.

[a]$p > 0.05$, no significant difference observed between placebo and FHE baseline, and placebo final.

[b]$p < 0.01$, FHE final compared between placebo final and FHE baseline.

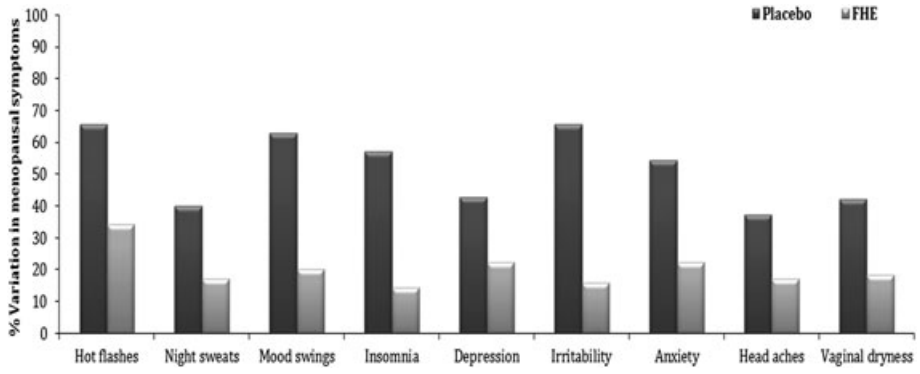

Figure 3. Effect of FHE supplementation on the postmenopausal symptoms of subjects as represented by the change in GCS score of participants on active and placebo at baseline and after 90 days.

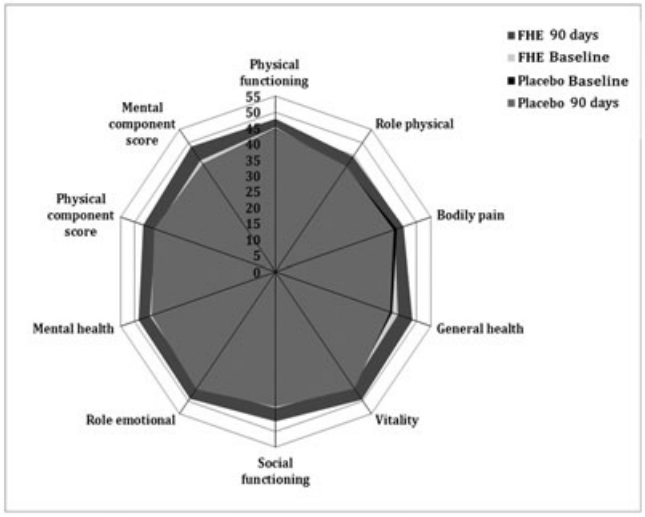

Figure 4. Spydergram summarizing the mean Short Form-36 Health Survey (SF-36®) domain scores of participants on FHE and placebo groups.

$p < 0.05$) when compared with the baseline. However, a similar increase was not found in the placebo group (Fig. 4).

Effect of fenugreek husk extract on biochemical and haematological parameters

Plasma estradiol levels of the FHE subjects showed more than twofold (120%) increase, as compared with the less than 5% increase in the placebo group [Fig. 5(a)]. The enhancement in estradiol level (from 131.22 to 288.46 pmol/L) was significant ($p < 0.01$) with respect to the baseline value and placebo group. There was also observed an enhancement of 2% in serum calcium levels among the participants in the FHE group as compared with a decrease of 0.8% in placebo group [Fig. 5(b)].

The present study involving a supplementation of 1000 mg FHE for 90 days did not produce any obvious signs of toxicity or adverse effects, as evident from the haematology and biochemical data in comparison with the placebo group (Table 3). The administration of FHE was found to reduce the total cholesterol, LDL and triglyceride levels in subjects with hypercholesterolemia and also helped to maintain the cholesterol levels in other subjects without any reduction in the HDL levels (Table 3). Other vital parameters like pulse and respiration were normal during study period as evident from the observations on 45th and 90th days of study. The blood pressure in supine and standing or upright positions was also within normal limits.

Effect of fenugreek husk extract on anthropometric measurements

Anthropometric measurements demonstrated that the participants in the present study were overweight with increased body fat percentage and waist circumference (Table 4). Overweight and obesity were present in 27% of the participants in the FHE group and 31% among the placebo group. Even though there was no significance ($p > 0.05$) observed in the BMI of the

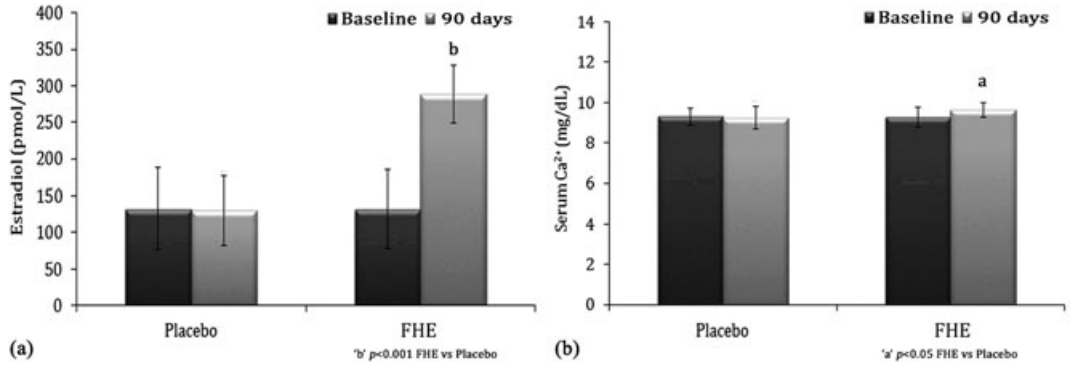

Figure 5. Variation of (a) estradiol and (b) serum calcium levels in postmenopausal women at baseline and after 90 days of treatments. [b]Δestradiol, $p < 0.001$ FHE versus placebo and [a]Δserum calcium, $p < 0.05$ FHE versus placebo.

Table 3. Effect of FHE on various haematological and biochemical parameters

	Placebo (n = 32)		FHE (n = 38)	
Parameters	Baseline	Final	Baseline	Final
Systolic BP (mmHg)	124.5 ± 20.9	125.2 ± 17.7[c]	126.9 ± 24.7[c]	125.8 ± 16.9[c,c]
Diastolic BP (mmHg)	79.7 ± 13.6	80.6 ± 11.2[c]	80.3 ± 16.5[c]	80.9 ± 12.8[c,c]
Hb (g/dL)	11.8 ± 2.32	12.02 ± 1.86[c]	11.53 ± 2.43[c]	12.65 ± 1.92[c,c]
RBS (mg/dL)	120.3 ± 13.64	118.9 ± 15.48[c]	116.4 ± 11.53[c]	114.8 ± 12.65[c,c]
Cholesterol (mg/dL)	223.2 ± 53.9	223.8 ± 51.7[c]	181.7 ± 58.29[a]	160.6 ± 69.10[b,a]
Triglycerides (mg/dL)	191.4 ± 91.58	186 ± 94.6[c]	196 ± 81.62[c]	190 ± 88.29[c,c]
HDL (mg/dL)	34.6 ± 16.84	36.49 ± 10.64[c]	54.6 ± 37.19[a]	57.6 ± 35.36[a,c]
LDL (mg/dL)	112.4 ± 3.39	113.7 ± 10.6[c]	82.9 ± 47.25[b]	75.2 ± 52.82[b,c]
VLDL (mg/dL)	38.3 ± 16.45	37.2 ± 18.83[c]	38.4 ± 24.32[c]	38.6 ± 20.59[c,c]

Data given as mean ± SD. No significant difference observed between placebo and FHE baseline, and placebo final ([c]$p > 0.05$).
[a]$p < 0.05$.
[b]$p < 0.01$.
[c]$p > 0.05$ when FHE final compared between placebo final and FHE baseline.

Table 4. Anthropometric characteristics of the FHE supplemented and placebo groups at baseline and after 90 days

	Placebo (n = 32)		FHE (n = 38)	
Parameters	Baseline	Final	Baseline	Final
Age (years)	52.97 ± 4.57	52.97 ± 4.57	53.31 ± 4.32	53.31 ± 4.32
Height (cm)	155.86 ± 6.67	155.86 ± 6.67	153.83 ± 4.90	153.83 ± 4.90
Weight (kg)	64.86 ± 10.21	65.06 ± 9.97	63.68 ± 6.71	61.31 ± 6.49
Body mass index (kg/m^2)	26.88 ± 4.99	26.97 ± 4.93	26.95 ± 2.99	25.98 ± 3.18
Mid-arm circumference (cm)	31.03 ± 2.83	31.17 ± 2.83	28.88 ± 2.04	27.34 ± 2.19
Waist circumference (cm)	103.26 ± 12.10	103.77 ± 11.09	95.57 ± 9.06	95.00 ± 8.47
Hip circumference (cm)	115.40 ± 8.33	115.03 ± 8.05	109.66 ± 9.59	107.00 ± 9.20
Waist:hip ratio	0.89	0.90	0.87	0.89

Data given as mean ± SD. No significant difference ($p > 0.05$) observed between placebo and FHE baseline, placebo final or when FHE final compared between placebo final and FHE baseline.

subjects supplemented with FHE, a trend towards a beneficial variation in body weight ($p = 0.06$), mid-arm ($p < 0.01$) and hip circumferences ($p = 0.08$) upon FHE powder supplementation was noted. However, no such variations were seen in the placebo group.

Adverse events

No adverse events were observed or reported among participants who received FHE powder or placebo at 1000 mg/day for 90 days.

DISCUSSION

The use of botanical and dietary supplements (BDS) among menopausal women has witnessed a tremendous growth in recent years. A survey on 500 perimenopausal and postmenopausal women conducted at the University of Illinois Medical Center has reported that 70% of women between the ages of 40 to 60 were using BDS as 'natural hormonal agents'. The congruency of traditional botanical medicines with the values, beliefs, lifestyles and safety of people was the main driving force for its widespread use (Geller *et al*., 2009; Pitkin, 2012). Soy foods and supplements containing phytoestrogenic isoflavones account the major share of use followed by black cohosh (Messina, 2014). Problems like vertigo, headache, nausea, vomiting, impaired vision and gastrointestinal issues have been found to be associated with the higher doses of black cohosh extract (Borrelli and Ernst, 2008). An increase in the incidence of metastasis was also observed when black cohosh was ingested in the diets of mice (Davis *et al*., 2008). Red clover, another botanical agent in use, has reported to possess no significant difference from placebo during long-term treatment (Geller *et al*., 2009). Nevertheless, another botanical agent of recent interest is hop extract with standardized levels of the prenylated isoflavone, 8-prenylnarignin, which was found to show significant estrogenic activities (Keiler *et al*., 2013), despite the recent reports on vaginal haemorrhage among hop extract used by postmenopausal women (van Hunsel *et al*., 2015).

Fenugreek is a relatively new botanical agent of immense interest to scientific research, although it has been widely used in India and China as a kitchen spice and home remedy for a number of health-related problems. Fenugreek seeds are rich source of proteins (20–23%), fat (5–6%), dietary fibre (40–45%) and phytochemicals including saponins (diosgenin, protodioscin, yamogenin), alkaloids (trigonellin), amino acids (4-hydroxyisoleucine), polyphenols and galactomannans (Nathiya *et al*., 2014; Yadav and Baquer, 2014). Many preclinical studies have demonstrated the safety and health beneficial pharmacological effects of fenugreek

seeds, most importantly the hypoglycaemic, hypolipidemic, gastroprotective and phytoestrogenic properties (Sreeja and Anju, 2010; Nathiya *et al*., 2014; Yadav and Baquer, 2014). Human clinical trials have also been reported on its beneficial effects in the management of primary dysmenorrhoea (Younesy *et al*., 2014), hot flashes and related menopausal discomforts (Hakimi *et al*., 2005; Hakimi *et al*., 2006; Abedinzade *et al*., 2015), breast milk-enhancing effects (galactogogue) (Reeder *et al*., 2013), libido enhancement (Rao *et al*., 2015) and for aphrodisiac effects (Steels *et al*., 2011).

In the present randomized double-blinded and placebo-controlled study, a standardized FHE was investigated for its estrogenic potential and ability to alleviate the postmenopausal discomforts in human subjects. The extract was produced by a hydroethanolic extraction, purification and formulation following Good Manufacturing Practice standards. Analyses of residual solvents, microbial levels (total plate count, yeast and mould, *Escherichia coli*, *Salmonella* and coliforms) and heavy metal ions (lead, arsenic, cadmium and mercury) have demonstrated its compliance to USP <561> requirements on articles of botanical origin. Acute (14 days) and subchronic (90 days) toxicity studies of FHE on Wistar rats showed no mortality or adverse effects without any significant changes in the haematological and biochemical parameters, with a no observed adverse effect level of 1000 mg/kg body weight/day (data not shown).

The analysis of the GCS and SF-36 questionnaires pertaining to the adverse climacteric symptoms and quality of life of the postmenopausal women participated in the present study demonstrated a significant improvement and positive effect of the novel formulation of FHE in the management of postmenopausal discomforts and hence in the quality of life. The individual GCS scores corresponding to the various domains of discomforts also revealed significant beneficial effect in alleviating climacteric symptoms, particularly the hot flashes, anxiety, depression, night sweats and vaginal dryness. The observed improvement in the quality of life of postmenopausal women belonging to the FHE group can be primarily attributed to its phytoestrogenic effect, as reported earlier (Younesy *et al*., 2014; Rao *et al*., 2015).

In the present study, the administration of FHE was found to be associated with a significant increase in estradiol levels. The rate of increase of estradiol among the individuals having varying baseline levels was found to be different, with a relatively low enhancement among those who are having high levels of baseline estradiol levels and vice versa. Although the mechanism of estradiol enhancement has not been investigated in the present study, the observed increase in estradiol and the improvement in the postmenopausal discomforts point towards the establishment of a healthy hormonal balance upon FHE supplementation. It has been demonstrated that the decrease in estrogen levels to below 60 pg/mL was the main reason for menopausal discomforts (Tepper *et al*., 2012). Estradiol also stimulates vaginal lubrication and blood flow, affecting a woman's capacity for sexual arousal and orgasm (Tepper *et al*., 2012). A recent clinical trial has also reported the libido-enhancing effect of fenugreek extract among healthy menstruating women (Rao *et al*., 2015). FHE supplementation was also found to have a beneficial effect in the management of healthy serum calcium levels (8.5 to 10.2 mg/dL). While an average enhancement in serum calcium levels was observed among the participants of the FHE group, the placebo group showed a decrease in serum calcium. Decrease in serum and bone calcium levels among postmenopausal women was very often reported among postmenopausal women leading to osteoporosis, which is a serious concern accounting 54% in USA (Andreopoulou and Bockman, 2015).

The lack of adverse effects or significant variation in the haematological and biochemical parameters of the participants in the FHE group demonstrates the safety of the supplementation of FHE at 1000 mg/day during the course of study. Haematopoietic system was generally considered as the most sensitive parameters that reflect the toxicity of any substance. Lipid profile of the subjects however showed a beneficial effect in reducing the total cholesterol and LDL levels in hyperlipidaemic subjects and helped to maintain the safe levels among those who had a normal range. It was already reported that the fenugreek extracts containing saponins possess hypolipidaemic effect (Roberts, 2011). Similarly, anthropometric measurements also remained with no significant variation, although a slight positive trend towards the reduction in body weight, mid-arm and waist circumferences was observed among the FHE-supplemented group. The percentage of overweight and obese subjects that remained 27 and 31% in the FHE and placebo group respectively even after 90-day supplementation shows that it is not conclusive to claim a beneficial effect of FHE on these parameters.

The efficacy of blinding was assessed by asking the participants and the investigator to guess to which group the patient was assigned. Eleven (34%) of the subjects in the placebo group and 16 subjects (42%) in the FHE group guessed their treatments correctly, while the investigator precisely ascertained the treatment assignment for 51% in the placebo and 58% in the FHE groups. Even then, it is assumed that the blinding was successful, because majority of the participants remained blinded as to the group allocation throughout the study period.

Despite the interesting results of the present pilot study, purposive sampling procedure, the short sample size, the absence of endometrial surveillance and lack of measurements of other key hormones (androgens and estrogens) involved in the menopausal phenomenon can be the limitations of the present study. Similarly, measurement of bone density, bone calcium levels and a questionnaire with respect to osteoporosis difficulties would have added more value to the present study.

CONCLUSIONS

Menopausal discomforts contributed by the hormonal imbalances are found to be common among women of all populations. Although it is not a disease, medical advices, medications and proper nutritional regimes have very often reported to be essential to manage this stage of life. Safe and natural botanical supplements, especially from the food components that are GRAS, were of emerging trend to manage postmenopausal discomforts. The present randomized placebo-controlled

study presented a novel formulation of FHE rich in phytoestrogens as a safe and natural alternative for menopausal symptom management. Supplementation of 1000 mg of FHE for 90 days was found to offer hormonal balance by significantly enhancing the estradiol levels, an estrogen that was shown to play a key role in menopausal discomforts. A significant enhancement in serum calcium and Hb levels was also observed among the subjects who consumed FHE. Despite the significant enhancement in lipid profile (total cholesterol, LDL and triglycerides) in the placebo group, FHE revealed a plausible cardiac beneficial effect by maintaining the lipid profile of the subjects in the baseline values. Moreover, FHE supplementation for 90 days was found to be safe with no adverse effects as shown by the haematological and serum biochemical parameters.

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Conflict of Interest

The authors disclose the following conflict of interest: FenuSMART™ is a patent pending and registered fenugreek husk extract formulation of M/S Akay Flavours & Aromatics Pvt. Ltd., Cochin, India. All human studies have been monitored and controlled by Nutrition Department at M/S Sri Jayadeva Institute of Cardiovascular Sciences and Research and University of Agricultural Sciences, Bangalore, under the supervision of Dr Jayalakshmi and Dr Shamshad Begum. Clinical signs, health conditions and haematology/biochemical parameters were measured at M/S Spurthy Diagnostic Centre, Bangalore, India under the supervision of Dr Shailaja. Dr M. A. Shankar, Dr Nutan and Dr C. N. Manjunath have no conflicts of interest. Dr KIM was involved in the protocol designing and critically evaluated the manuscript.

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