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FULL ARTICLE

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Efficacy of a novel extract of fenugreek seeds in alleviating vasomotor symptoms and depression in perimenopausal women: A randomized, double-blinded, placebo-controlled study

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Abstract

The present randomized, double-blinded, placebo-controlled study investigated the effect of a standardized fenugreek extract (FHE) on perimenopausal discomforts and its influence on hormonal balance and safety. Healthy women characterized with perimenopausal symptoms ($n = 48$), as assessed by MRS questionnaire, were randomized either to FHE ($n = 24$) or placebo ($n = 24$) and supplemented with 250 mg × 2/day for 42 days. Both inter and intra-group comparison revealed a significant improvement in somatic, psychological, and urogenital scores in FHE group, especially for hot flashes (25.9%), night sweats (26.5%), depression (31.8%), and insomnia (21.6%). Further hormone analysis revealed an enhancement in serum estradiol (18.9%), free testosterone (38.2%), and progesterone (19.9%) concentrations and a significant decrease in FSH (38.2%) and SHBG (21.1%) concentrations toward establishing a hormonal balance among FHE-group; without significant changes in other clinical safety parameters. Thus, FHE supplementation offered a significant reduction in vasomotor effects and depression in perimenopausal women, without any adverse effects

Practical applications

Fenugreek is a popular kitchen spice and Ayurvedic medicine for a variety of health conditions including diabetes, hypercholesterolemia, hepatotoxicity, gastritis, and also for a variety of hormone-related health conditions such as sexual functions, lactation, osteoporosis, PCOS, and post/perimenopausal discomforts. Fenugreek is rich in alkaloids, steroidal saponins, flavonoids and 4-hydroxyisoleucine. The present randomized-controlled study investigated the plausible application of a standardized hydro-ethanolic extract of fenugreek seeds (FHE) having a unique 3:1 ratio for protodioscin to trigonelline in the management of perimenopausal discomforts. It was observed that FHE at a dosage of 250 mg × 2/day for 42 days significantly reduced the discomforts, especially vasomotor symptoms and depression, and helped to attain a hormonal balance without any adverse effects or deviations in clinical safety

parameters. Thus, FHE could be a potential natural agent for the management of post and perimenopausal discomforts and has to be explored in future studies.

KEYWORDS

depression, fenugreek, hormonal balance, hot flashes, perimenopause, *Trigonella foenum-graecum*

1 | INTRODUCTION

The World Health Organization and the North American Menopause Society defines perimenopause (menopausal transition) as the time period preceding natural menopause (usually 2 to 8 years) and 1 year following final menses (WHO, 1996). The physiological and hormonal changes during the perimenopausal phase affect the quality of life of women, even with a devastating impact on the mental health. The Penn Ovarian Aging Study, a cohort study, showed significant increase in depressive symptoms during the menopausal transition (Freeman et al., 2004). The "Harvard Study of Moods and Cycles" had examined the association between the menopausal transition and the onset of the first episode of depression and suggested that women having no lifetime history of depression have a significant risk for the onset of depression during the earlier phase of menopausal transition (Cohen et al., 2006). Generally, women who experience depressive and anxiety symptoms for the first time in their mid-40s are actually experiencing depression related to the perimenopausal phase (Kulkarni, 2018). Hot flushes, night sweats, sleep disturbances, mood swings, and fatigue are the other most commonly reported issues during perimenopausal transition (Cheung et al., 2004).

Constant changes in reproductive hormones occur in a woman's life; a decline in estradiol (E2) and elevation in follicle stimulating hormone (FSH) was reported during perimenopause (Santoro et al., 2015). It is the hormonal imbalance which trigger a variety of acute discomforts, known as menopausal discomforts. For instance, perimenopause-related changes in estradiol may interact with low levels of dihydroxyepiandrosterone (DHEA) in some women to increase their vulnerability to develop depression (Schmidt et al., 2002). Hormone replacement therapy (HRT) is generally recommended as the first line of treatment for both perimenopause and postmenopausal discomforts. However, attempts with natural aids and alternative treatment methods are gaining more acceptance throughout the world (Cheung et al., 2004; Kargozar et al., 2017). Regular exercise, yoga, meditation, and dietary advice along with education on menopause have been shown to be of help in the management of menopausal discomforts (Kulkarni, 2018).

Standardized extracts of Soy (*Glycine max*), Red clover (*Trifolium pratense*), Black cohosh (*Actaea racemosa*), Chasteberry (*Vitex agnus-castus*), Asian ginseng (*Panax ginseng*), and Hops (*Humulus lupulus*) have been in use for the management of postmenopausal discomforts (Kargozar et al., 2017). Fenugreek seeds are popular kitchen spice and Ayurvedic medicine for a variety of health conditions including diabetes, hypercholesterolemia, hypertriglyceridemia, hepatotoxicity, and gastritis (Fuller & Stephens, 2015). Fenugreek has also been found to be useful for a variety of hormone-related health functions such as sexual functions, lactation, osteoporosis, PCOS, and post/perimenopausal discomforts (Abedinzade et al., 2015; Hakimi et al., 2005; Hassanzadeh Bashtian et al., 2013; Kaviarasan et al., 2007). Fenugreek extract has been shown to have estrogenic activity and binds to estrogen receptors (Sreeja et al., 2010). It is rich in alkaloids, steroidal saponins, flavonoids, and a non-proteinogenic amino acid 4-hydroxyisoleucine (Wani & Kumar, 2018). Trigonelline is considered as the most important pharmacologically relevant metabolite of fenugreek, having beneficial effects even against liver and cervical cancer (Bahmani et al., 2016). Protodioscin is a major saponins in fenugreek seeds which has been shown to convert to DHEA, a precursor for estrogen (Pavin et al., 2018). 4-hydroxyisoleucine has been shown to be insulinotropic (Avalos-Soriano et al., 2016). An earlier study employing a proprietary extract containing protodioscin and trigonelline in the ratio of 3:1 (w/w) (FenuSMART®; *hereinafter referred to as "FHE"*) had revealed a significant beneficial effect on postmenopausal discomforts when supplemented at 500 mg × 2/day for 90 days (Shamshad Begum et al., 2016). Thus, we hypothesized that a low dosage of FHE would ameliorate the clinical symptoms among perimenopausal participants. Thus, the current study investigated the influence of FHE on perimenopausal discomforts and hormonal balance when supplemented at 250 mg × 2/day for 42 days.

2 | MATERIALS AND METHODS

2.1 | Study design and recruitment

The present study was conducted in a randomized, double-blinded, placebo-controlled, and parallel group design. Sixty women participants within the age of 39–51 years who visited Aman Hospital and Research Center, Gujarat, India for medical consultancy were screened on the initial visit (visit 1). A total of 48 women in perimenopausal stage (as per STRAW Classification) (Harlow et al., 2012) with ≥7 day's difference in length of consecutive cycles or late perimenopause women with amenorrhea for ≥60 days were selected. Also, participants with FSH level of >25 IU/L and experiencing hot flash score of 10 or higher for one week before the visit 1 were also included as a selection criterion in the current study. Participants were identified by purposive sampling under the supervision of a qualified Gynecologist and Nutritionist.

The detailed inclusion and exclusion criteria are listed in Table 1. A minimum of 48 participants were required to achieve a statistical power of 80% on the basis of a 20% improvement in symptoms as measured by the total domain score of the MRS questionnaire. All the participants underwent a medical screening comprising anthropometric measurements and were asked to answer the questionnaire related to health, medication, occupation, and physical activity. All participants were informed about the nature and risks of the experimental procedures and their written informed consent were obtained before the study. Participants were asked to stay away from fenugreek-containing diet during the study period. The study was approved by Institutional Ethics Committee, Aman Hospital and Research Centre and is in accordance with the clinical research guidelines of Government of India and was registered in the clinical trial registry of India (CTRI/2018/09/015614 dated 05/09/2018).

The natural process of menopause, hormonal changes and perimenopausal discomforts were detailed to the participants and a written consent was obtained from all individuals who were willing to take part in the study after explaining the objective of the study. Baseline sociodemographic variables (age, race, civil status, marital status, education, and age of menopause) were also noted.

TABLE 1 Inclusion and exclusion criteria of the study participants

Inclusion criteria
1. Age 39–51 years (both inclusive)
2. Perimenopausal Women according to STRAW Classification. Early perimenopause women with ≥7 days difference in length of consecutive cycles or late perimenopause women with amenorrhea for ≥60 days
3. FSH level of >25 IU/L and experiencing daily hot flash score of 10 or higher for 1 week before the visit 1
4. Participants with a BMI of ≤30 kg/m^2
5. Participants with normal fasting blood glucose level
6. Participants understands the study procedures and provides signed informed consent to participate in the study
7. Clinical screening including vital signs and laboratory evaluations (including hematology, biochemical, and hormonal) within the reference range for the testing laboratory or the results are deemed not clinically significant for inclusion into this study by the investigator
Exclusion criteria
1. History of cerebrovascular disease, heart attack, or angina at any time or on anticoagulant or antiplatelet drugs on a daily basis for any conditions
2. Previous treatment with hormonal treatment or fenugreek derived products in the previous 12 months
3. History of using estrogen or progestin-containing products in the past 3 months of recruitment
4. Chronic or acute life stressors relating to a major life change, experiencing depression, and/or receiving medication for such illness or disorders, receiving statins or other drugs known to impact on steroid hormone levels
5. History of breast, endometrial, other gynecological cancer at any time or other cancer within the last 5 years
6. Participants with abnormal ECG, biochemical, or hematological values
7. Participants, who are smokers, tobacco, or alcohol user
8. Use of any recreational drugs (cocaine, amphetamine, barbiturates, benzodiazepines, cannabinoids, and morphine)
9. History of clinically significant illness or any other medical disorder that may interfere with participants treatment, assessment, or compliance with the protocol
10. Participated in a clinical study with an investigational drug or biologic within the last 30 days
11. Any condition that in opinion of the Investigator, does not justify the participants participation in the study

2.2 | Intervention

The proprietary hydro-ethanolic extract of fenugreek seeds (FHE, *patented and registered as FenuSMART®*) was obtained from Akay Natural Ingredients, Cochin, India. The extract of fenugreek seeds was prepared by hydro-ethanolic extraction, concentration and spray drying process as a free flowing granular powder with composition of 10.3% protodioscin, 3.1% trigonelline, and 42.6% total saponin content. Food-grade microcrystalline cellulose (MCC) powder was employed as the placebo. Both FHE and placebo were packed in an identical, hard shell gelatin capsule were analyzed for the quality requirements as per U.S. Pharmacopeia for dietary supplements (Sureshkumar et al., 2018).

2.3 | Randomization and blinding

Participants in the particular study were randomly allocated to two intervention arms (Block size = 2) by permuted-block randomization using computer generated allocation table to receive either FHE ($n = 24$) or placebo ($n = 24$). The contract research organization staff (coordinator) who were not involved in the screening of participants handled the randomization sequence schedule. The participants and the investigator (medical doctor) were blinded to the intervention group while allocation list was maintained by the study coordinator.

Sequentially numbered airtight identical bottles containing hard shell gelatin capsules containing 250 mg of either FHE or placebo were provided on visit 2 (day 1), and visit 3 (day 28) and were instructed to take one capsule [(250 mg × 1) after breakfast and another one after dinner (250 mg × 1) for a total of 42 days. The degree of adherence and compliance of the participants was assessed by "count pills" strategy. A Consolidated Standards of Reporting Trials (CONSORT) flow diagram describing the number of participants screened, randomized, withdrawn, and completed the study is shown in Figure 1a. The details of daily drug administrations and side effects or discomforts (if any), were monitored on a weekly basis through regular telephonic follow-ups. The details of the analyses performed during each visits of the study period were as shown in Figure 1b.

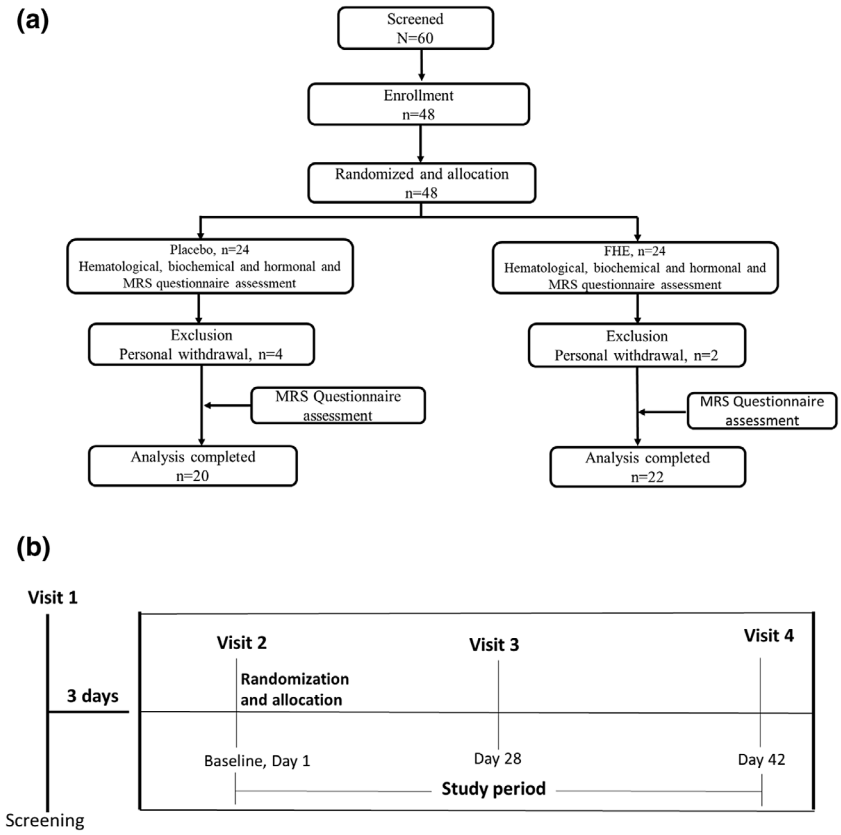

FIGURE 1 (a) CONSORT flow diagram of study, (b) Trial Schedule showing the number of visits during the 42 days of study

2.4 | Outcome measures

The primary outcome includes the efficacy evaluation of supplementation of FHE on perimenopausal symptoms as measured by menopause rating scale (MRS) questionnaire on day 1, day 28 and day 42. MRS is a health-related Quality of life scale developed to measure the severity of menopause related symptoms. The scale consists of 11 items, self-completed by the participants at baseline (day 1) and at the end of study (day 42). There are three dimensions of symptoms: somatic (1–3, 11), psychological (4–7), and urogenital factor (8–10). The severity of complaints of each items are explained in a 5-point rating scale (score of 0—no complaint and 4—very severe symptom). The composite scores for each of the dimensions (sub-scales) are based on adding up the scores of the items of the respective dimensions and the total score is the sum of the dimension scores (Heinemann et al., 2004).

The secondary outcome includes the evaluation of the hormonal balance and safety assessment upon supplementing with FHE and comparing it with placebo. Blood samples were collected by vein puncture at baseline (Day 1) and at the end of study (day 42). Samples were taken into EDTA/non-EDTA vials for assaying hematological and biochemical parameters. Serum was separated from the collected blood samples by centrifugation at 6,000 rpm at 4°C for 10 min and stored at −80°C for further analysis. The hormone analysis in serum samples were carried out using ELISA kit methods. Human FSH and testosterone kits were obtained from Abcam, UK; sex hormone binding globulin (SHBG) and estradiol ELISA kits were from Biocompare, USA; and progesterone kit was from Cayman Chemicals, USA. For safety assessment, the hematological parameters were assessed using an autoanalyzer (Meril Biochemistry analyzer, Eris diagnostics, and Instruments, India). Biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum creatinine, fasting blood sugar (FBS), lipid profiling (total cholesterol, HDL cholesterol, LDL cholesterol, VLDL and triglycerides) and creatinine markers were analyzed with assay kits provided by M/s Agappe Diagnostics Pvt. Ltd. Bangalore, India.

2.5 | Statistical evaluation

Statistical analyses were carried out using the statistical Package (SPSS Inc. Chicago, IL, USA) version 25.0. Independent sample *t* tests was used for group comparison (FHE group vs. control group) of demographic baseline characteristics and also for MRS questionnaire. The potential changes in hormone levels, hematological, and biochemical parameters between placebo and FHE supplemented groups was evaluated using a 2×2 repeated measures (RM) analysis of variance with Bonferroni post hoc corrections. The compliance of the FHE and placebo intake was compared using *t* test for equality. The results were presented as mean $\pm$ *SD* and p value $\leq .05$ was considered to be significant.

TABLE 2 Baseline demographic characteristics of the study participants

Characteristics	Placebo ($n = 20$)	FHE ($n = 22$)
Age (years)	45.10 ± 3.7	45.3 ± 3.8
Weight (kg)	60.79 ± 5.16	59.83 ± 5.2
BMI (kg/m^2)	23.55 ± 1.7	22.44 ± 1.8
Education	*n*%	*n*%
Below high school	5(25)	5(22.7)
High school and above	15(75)	17(77.2)
Marital status		
Married	17(85)	16(72.7)
Divorced	2(10)	3(13.6)
Widow	1(5)	3(13.6)
Residence		
Urban	12(60)	16(72.7)
Rural	8(40)	6(27.2)
Menstrual status		
Polymenorrhea	5(25)	4(18.2)
Oligomenorrhea	4(20)	6(27.3)
Amenorrhea	11(55)	12(54.5)
MRS		
Total score	12.9 ± 2.9	12.45 ± 2.9
Somatic score	5.5 ± 1.4	5.2 ± 1.6
Psychosocial score	5.1 ± 1.5	4.8 ± 1.6
Urogenital score	2.3 ± 1.3	2.4 ± 1.1

Note: Values are expressed as mean ± *SD*.

Abbreviations: BMI, body mass index; MRS, menopause rating scale.

3 | RESULTS

3.1 | Study participants and baseline characteristics

Out of the 48 perimenopausal participants enrolled in the study, only 42 participants successfully completed the study. Four participants from placebo and two participants from FHE group withdrawn from the study due to personal reasons. No difference was observed in the supplementation compliance between the groups (98.4% in placebo and 98.6% in FHE, $p = .841$). A total of 20 participants in placebo and 22 participants in FHE completed the study as shown in Figure 1a,b.

Baseline demographic characteristics were statistically insignificant. The sociodemographic variables of the participants were presented in Table 2. The mean age of participants were 45.1 ± 3.5 and 45.3 ± 3.8 years in placebo and FHE, respectively. The mean baseline body weight of placebo and FHE were 60.79 ± 5.16 and 59.83 ± 5.2, respectively. The baseline BMI in placebo was 23.55 ± 1.73 and that of FHE was 22.44 ± 1.85. The average total MRS scores of placebo and FHE at baseline were 12.9 ± 2.9 and 12.45 ± 2.9, respectively.

3.2 | Efficacy of FHE supplementation on menopausal symptoms (MRS)

The efficacy of treatment was evaluated by the reduction in MRS scores at baseline (day 1), day 28 and day 42 as shown in Figure 2. At the baseline, the MRS total score was 12.9 ± 2.9 for placebo and 12.45 ± 2.9 for FHE group. On 28th day, the total score was reduced

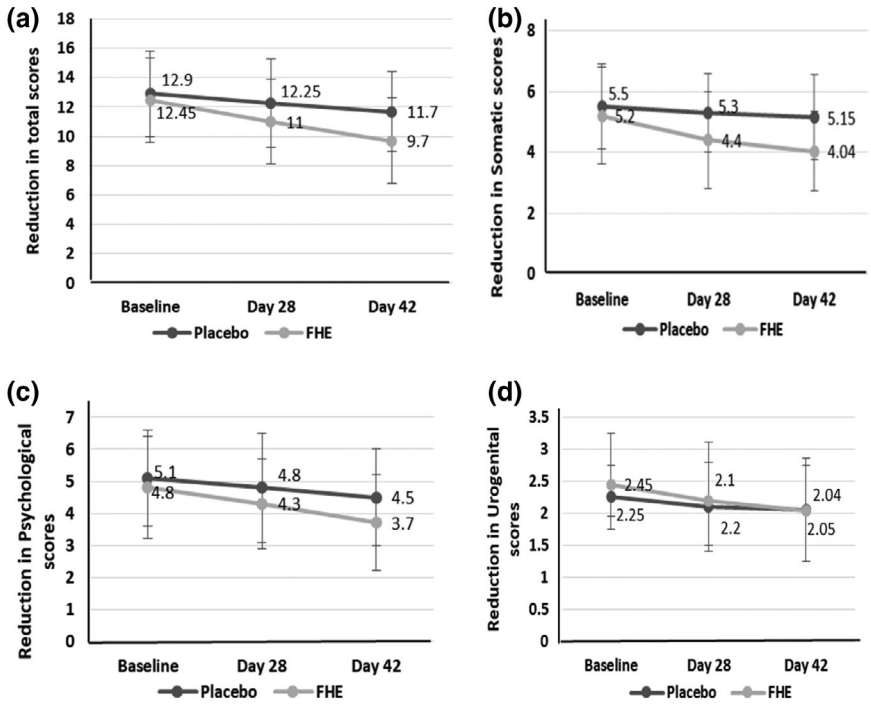

FIGURE 2 Reduction in total, somatic, psychological, and urogenital symptoms scores by day 28 and day 42 in placebo and FHE groups. Mean values are expressed in brackets in figure. *$p \leq .05$ was considered to be significant

to 12.2 ± 3.0 (5.4%) in placebo and 11.0 ± 2.9 (11.6%) in FHE group. The trend continued and by the end of the study period (Day 42), the MRS total score was reduced to 11.7 ± 2.7 (9.3%) in placebo and 9.7 ± 2.8 (22.1%) in FHE group.

There was a gradual reduction in the occurrence and severity of somatic, psychological and urogenital symptoms after 28 days of FHE supplementation [4.3 ± 1.5, (14.9%), 4.4 ± 1.4 (10.4%) and 2.2 ± 1.1 (8.3%), respectively] when compared to placebo [5.3 ± 1.3 (3.6%), 4.8 ± 1.7 (5.8%) and 2.1 ± 1.5 (4.5%), respectively]. The scores further reduced to 4.0 ± 1.2, 3.7 ± 1.6, and 2.0 ± 0.45 (21.8, 22.9, and 18.36%, respectively, for somatic, psychological, and urogenital symptom scores in FHE group as compared to the 5.5, 11.7, and 6.8% reduction in placebo. Among the various individual symptom scores, hot flashes, night sweats, depression, and insomnia showed the most ($p \leq .05$) notable improvements, with 20.3, 15.6, 11.4, and 10.8%, respectively, in FHE group when compared to the placebo (2.1, 5.2, 2.4, and 5%, respectively) on day 28. On day 42, the improvement increased to 25.9, 26.5, 31.8, and 21.6%, respectively, in FHE group and 8.3, 7.5, 7.1, and 7.5%, respectively, in placebo (Figure 3).

3.3 | Effect of FHE on hormone balance

The estradiol levels were significantly increased (18.3%), progesterone (19.9%), total testosterones (19.5%), and free testosterone (38.2%), were seen at the end of the study in FHE supplemented group as compared to the placebo (6.8, 8.0, 6.25, and 10% respectively). The FSH (17.3%) and SHBG (21.1%) levels were significantly lowered upon FHE treatment when compared to placebo which showed 4.3 and 7.9% reduction respectively (Table 3).

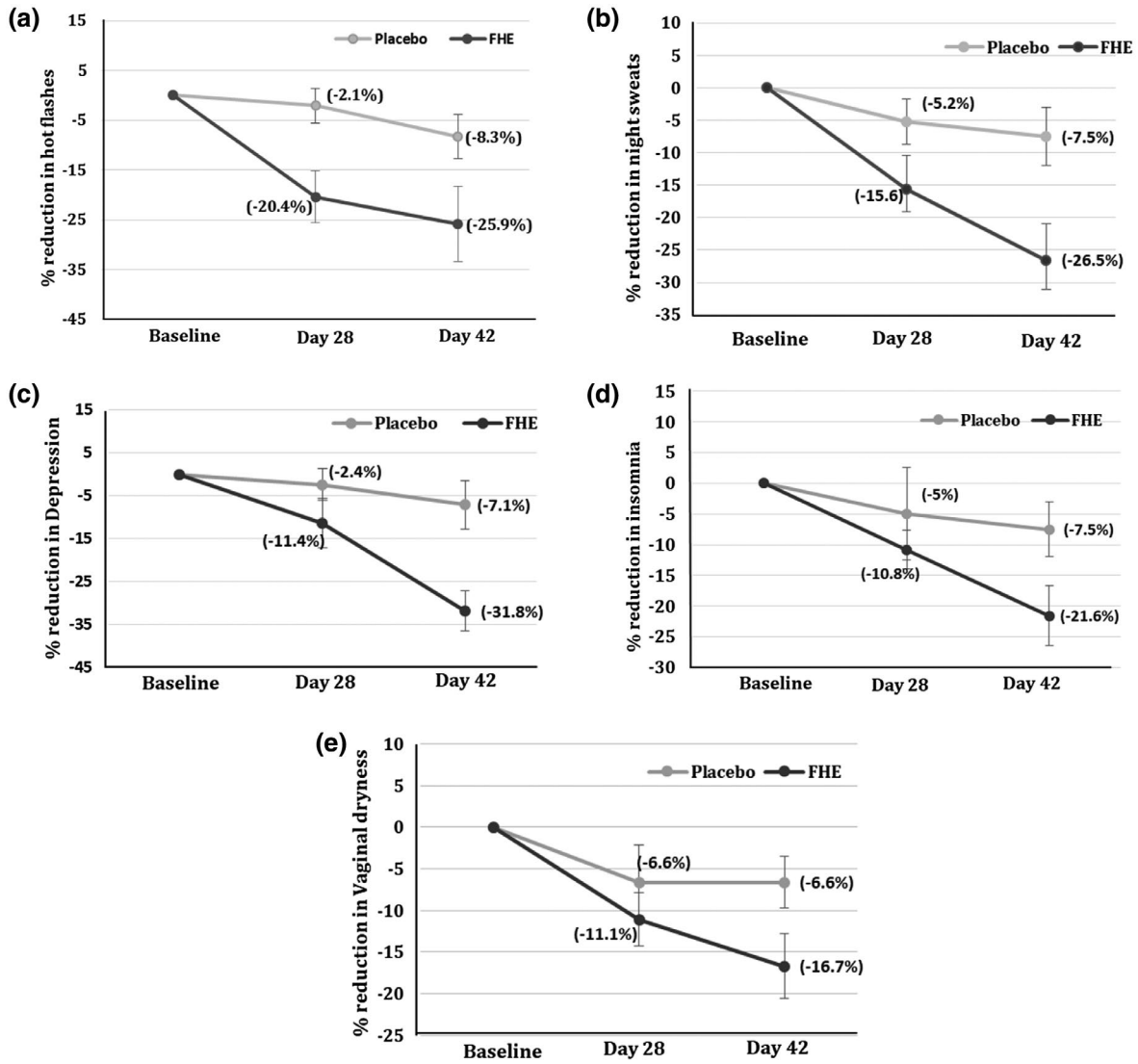

FIGURE 3 Percentage reduction in individual MRS sores. (a) Hot flashes, (b) Night sweat, (c) Depression, (d) Insomnia, and (e) Vaginal dryness scores in placebo and FHE supplemented perimenopausal women at day 28 and day 42. Values are expressed as percentage reduction of total individual symptoms scores of placebo and FHE participants

3.4 | Safety and tolerance of FHE supplementation

The fenugreek seed extract, FHE, was found to be well tolerated during the study period. The safety assessed by hematological and biochemical parameters showed that there was no significant changes in these parameters at baseline or at the end of the study upon FHE treatment, when compared with placebo (Table 4). No adverse events or side effects were also reported during the study period.

4 | DISCUSSION

The present study reported the beneficial effect of a standardized hydro-ethanolic extract of fenugreek seeds in alleviating the perimenopausal discomforts when supplemented at 250 mg × 2/day for 42 days. The study showed a significant improvement in various symptoms, especially on vasomotor symptoms and depression pointing toward the phytoestrogenic effects of FHE. Further, the hormonal analysis confirmed the influence of FHE toward the achievement of a balanced hormonal concentration within the safe range.

Though there are many studies examining the postmenopausal discomforts, only a few have dealt with the perimenopausal discomforts. Maintaining a proper hormone balance during the menopausal transition phase will help in the management of perimenopausal discomforts (Santoro et al., 2015). Transition to menopause and its changing hormonal milieu are strongly associated with the onset of a depressed mood even among women with no history of depression (Freeman et al., 2006). The positive association between vasomotor symptoms and depression in both community dwelling menopausal women and women presenting to clinical services has been reviewed by Worsley et al., (2014). The present contribution represents the first double-blinded randomized placebo-controlled clinical study to evaluate the efficacy of a fenugreek extract on perimenopausal discomforts, especially on hormonal imbalance and vasomotor symptoms. Fenugreek extract used in the present study is rich in protodioscin and trigonelline, the two important phytochemicals that are reported to have estrogenic effect. Previously, supplementation of fenugreek seed powder (6 g/day for 8 weeks) was shown to relieve early menopausal symptoms especially hot flashes (Hakimi et al., 2006). Our earlier study with 500 mg × 2/day of FHE has also been shown significant beneficial effect on postmenopausal women (Shamshad Begum et al., 2016). A recent study has also shown significant improvement among postmenopausal discomforts when supplemented with 250 mg × 2/day of FHE for 42 days (unpublished data, communicated). Another study states that fenugreek seed flavonoids exhibit an antidepressant-like effect by downregulating the KLF11/SIRT1-MAO-A pathway, inhibiting MAO-A expression and activity, as well as upregulating monoamine neurotransmitters levels (Wang et al., 2019).

Most women experience moderate to severe vasomotor symptoms like hot flashes and night sweats during the menopausal transition (Woods & Mitchell, 2005). A decline in estrogen production alter the neurotransmitter activity thereby decreasing serotonin levels, leading to the decreased density of hypothalamic serotonin receptors (5-HT_{2A}) responsible for thermoregulation and such changes may narrow the threshold level of the thermoregulatory zone, leading to hot flashes (Joswig et al., 1999; Rybaczyk et al., 2005). The consumption of phytoestrogens can alter the neuroendocrine mechanism of core body temperature regulation and reduce hot flashes (Hairi et al., 2019). Phytoestrogens preferentially bind to estrogen receptor-β (ER-β) and ER-β activation results in 5-HT_{2A} upregulation (Rybaczyk et al., 2005). In the current study, the improvement in hot

TABLE 3 Hormone levels of placebo and FHE supplemented groups

	Placebo		FHE supplemented group		
Parameter	**Baseline**	**End of study**	**Baseline**	**End of study**	***p*-Value**
Estradiol (pmol/L)					
Reference range: <60 pmol/L	43.46 ± 8.2	46.44 ± 8.7	44.77 ± 7.7	52.96 ± 8.3	.001
Progesterone (ng/ml)					
Reference range: <1.0 ng/ml	0.50 ± 0.14	0.54 ± 0.12	0.55 ± 0.17	0.66 ± 0.15	.037
Follicle stimulating hormone (IU/L)					
Reference range:<94 IU/L	47.30 ± 8.03	45.27 ± 7.6	49.45 ± 15.7	40.88 ± 12.5	.001
Total testosterone (ng/ml)					
Reference range: 0.2–1.2 ng/ml	0.16 ± 0.04	0.17 ± 0.03	0.17 ± 0.05	0.20 ± 0.05	.034
Free testosterone (pg/ml)					
Reference range: 0.1–1.7 pg/ml	0.10 ± 0.019	0.11 ± 0.024	0.11 ± 0.02	0.15 ± 0.02	.002
SHBG (nmol/L)					
Reference range: 16–128 nmol/L	56.74 ± 10.65	52.21 ± 11.75	57.5 ± 13.08	45.38 ± 10.6	.001

Note: Values are expressed as Mean ± *SD*. *p*-values shown was the significance of FHE from baseline to end of study. $p \leq .05$ is considered as significant.

Abbreviations: CTX-1, C-terminal telopeptide; SHBG, sex hormone binding globulin.

TABLE 4 Hematological and biochemical parameters of FHE and placebo participants

Parameters	Groups	Baseline	End of Study	*p*-Value
Hemoglobin (g/dl)	Placebo	14.8 ± 0.39	14.7 ± 0.26	.402
	FHE	14.76 ± 0.89	14.43 ± 0.95	
Total leukocyte count (cells/cumm)	Placebo	6,357.5 ± 324.3	6,686.5 ± 132.3	.959
	FHE	6,381.2 ± 510.8	6,719.7 ± 425.3	
Total RBC count (million/cumm)	Placebo	4.52 ± 0.11	4.24 ± 0.13	.103
	FHE	4.72 ± 0.48	4.82 ± 0.64	
PC (lakhs/cumm)	Placebo	2.14 ± 0.36	2.36 ± 0.43	.458
	FHE	2.31 ± 0.42	2.41 ± 0.38	
PCV (%)	Placebo	35.2 ± 1.3	36.7 ± 1.7	.328
	FHE	33.6 ± 1.4	35.4 ± 1.5	
MCH (pg)	Placebo	31.30 ± 1.4	30.49 ± 1.2	.377
	FHE	31.78 ± 1.4	30.31 ± 1.3	
MCHC (g/dl)	Placebo	31.2 ± 1.2	33.75 ± 1.4	.432
	FHE	33.56 ± 1.1	32.45 ± 1.2	
MCV (fL)	Placebo	91.82 ± 2.40	93.44 ± 2.30	.408
	FHE	92.55 ± 2.29	93.53 ± 1.63	
Neutrophils (%)	Placebo	54.40 ± 3.2	54.69 ± 3.2	.887
	FHE	53.22 ± 3.03	53.75 ± 2.73	
Lymphocytes (%)	Placebo	39.30 ± 2.0	38.5 ± 3.0	.778
	FHE	40.45 ± 3.3	39.2 ± 3.4	
Eosinophils (%)	Placebo	4.19 ± 0.8	4.09 ± 0.8	.462
	FHE	4.00 ± 0.5	4.20 ± 0.7	
Monocytes (%)	Placebo	3.43 ± 0.69	3.23 ± 0.60	.166
	FHE	3.32 ± 0.61	3.53 ± 0.71	
ESR (mm/hr)	Placebo	10.4 ± 1.2	10.3 ± 1.1	.255
	FHE	10.5 ± 1.2	9.8 ± 1.2	
Triglycerides (mg/dl)	Placebo	141.4 ± 4.9	142.6 ± 5.9	.129
	FHE	143.7 ± 4.6	141.1 ± 5.6	
Total Cholesterol (mg/dl)	Placebo	201.5 ± 8.19	204.7 ± 9.5	.735
	FHE	201.5 ± 9.03	203.1 ± 7.7	
HDL Cholesterol (mg/dl)	Placebo	43.50 ± 2.6	45.26 ± 2.7	.905
	FHE	45.49 ± 3.2	47.03 ± 3.4	
LDL Cholesterol (mg/dl)	Placebo	160.3 ± 5.4	165.5 ± 5.1	.129
	FHE	163.6 ± 4.1	164.6 ± 6.4	
VLDL Cholesterol (mg/dl)	Placebo	31.33 ± 4.2	33.32 ± 3.3	.486
	FHE	31.01 ± 3.7	34.45 ± 4.7	
SGOT (U/L)	Placebo	24.04 ± 4.4	26.63 ± 3.9	.757
	FHE	29.67 ± 9.6	31.11 ± 9.6	
SGPT (U/L)	Placebo	37.29 ± 7.5	41.59 ± 8.7	.287
	FHE	40.15 ± 6.7	40.67 ± 4.3	
Alkaline phosphatase (IU/L)	Placebo	50.5 ± 7.2	54.8 ± 7.7	.635
	FHE	65.5 ± 9.0	68.0 ± 9.6	

Note: A general linear model with adjustments for baseline values were used to predict marginal means (95% CI) for each outcome variable. Values are expressed as mean ± *SD*. *p*-Values shows the significance of FHE from baseline to end of study. $p \leq .05$ is considered as significant.

Abbreviations: ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PC, platelet count; PCV, packed cell volume; RBC, red blood cells; SGOT, serum glutamate oxaloacetate transferase; SGPT, serum glutamate pyruvate transferase; VLDL, very low-density lipoprotein.

flashes, night sweats, and insomnia might have reflected from the estrogen like action of phytoestrogens present in fenugreek (Hakimi et al., 2005).

The reproductive hormone changes during menopause causes many discomforts which can indirectly lead to depression. A survey has showed the association of depression with sexual dysfunction (Bonierbale et al., 2003) and this often results in women undertaking antidepressants. Antidepressant medications can further exacerbate the difficulty with sexual function in the domains of sexual desire, arousal, and orgasm (Lorenz et al., 2016). The hot flashes lead to sleep disturbance and then to daytime fatigue, poor quality of life, mood disturbance, and then depressive symptoms (Freeman et al., 2006). The decreased density of 5-HT_{2A} and lower activity of serotonin during low estrogen levels leads to depression (Berton & Nestler, 2006). Reports show the potential of estrogens in increasing the serotonin levels (Bethea et al., 1999; Blum et al., 1996). Moreover, saponins as well as fenugreek seed flavonoids were reported to increase the levels of monoamine neurotransmitters such as serotonin in the brain (Abbas et al., 2013). Thus, the antidepressant activity of FHE could be attributed to its estrogen enhancing property thereby improving the levels of serotonin and the activity of 5-HT_{2A}. Also, supplementation of 4-hydroxyisoleucine from fenugreek seeds had shown antidepressant and antianxiety effects on olfactory bulbectomized rats (Kalshetti et al., 2015) and was also found to ameliorate cognitive deficits in streptozotocin-induced diabetic rats (Kodumuri et al., 2019). Since, there is a strong association between insomnia and depression (Nutt et al., 2008), the improvement in depression, in turn, led to efficient sleep in the current study.

Another effect of low estrogen levels is the changes in the vaginal wall which is often associated with sexual discomfort and pain (Bachmann & Leiblum, 2004). FHE supplementation improved vaginal dryness in perimenopausal women. The phytoestrogenic property of FHE might have helped in relieving the symptom. Estrogen is known to maintain the fluid film that lines the vaginal walls, contributing to tissue elasticity and thus relieving vaginal dryness and improving the sexual response (da Silva Lara et al., 2009). Recent trials have also suggested that oral and vaginal preparations of DHEA can improve vaginal dryness (Labrie et al., 2016).

The menopause transition characterized by loss of ovarian follicular activity leads to an imbalance in the hormone levels in perimenopausal women (Burger et al., 2002). The reproductive hormone levels were balanced upon FHE supplementation in the current study. Supplementation of hydro-alcoholic extract of fenugreek in mice has shown to improve the reproductive hormone levels in mice (Modaresi et al., 2012). The increase in estradiol and testosterone found in the study might be due to the presence of protodioscin in FHE, a steroidal saponins compound and can be converted to DHEA (Adimoelja, 2000). DHEA is then converted to testosterone by 17β-hydroxysteroid dehydrogenase (17β-HSD). The study on administration of low doses (25 mg) of DHEA positively modulated several endocrine parameters in early and late postmenopausal women, inducing the increase of the androgenic, estrogenic, and reducing the climacteric symptoms, similarly to estrogen replacement therapy (Genazzani et al., 2003). Also, women with higher DHEA have reported greater overall quality of life, physical functioning, and fewer depressive disorders (Santoro et al., 2005). Moreover, DHEA and testosterone can also be aromatized to estrogen in the ovary through steroidogenic enzyme (Zhou et al., 2015). Thus, the presence of protodioscin in fenugreek might have contributed to balanced hormone levels in the FHE participants.

There was an increase in both total and free testosterone levels in FHE supplemented group which can be correlated with a decrease in SHBG levels. The circulating testosterone is bound to SHBG and the bound form is considered inactive. The SHBG levels are seen to increase with age, however, the supplementation of FHE decreased the SHBG level in perimenopause participants. Diet has an influence on SHBG levels, a study has reported a decreased SHBG level upon supplementation with high fiber diet in premenopausal women (Goldin et al., 1994).

Well-defined protocol and replicability can be considered as the strength of the present study. However, the small sample size ($n = 48$), and the shortage in the number of study completed participants remains as a limitation of the study. The small sample size may be rationalized on the principle that the pilot human studies to guide the future research can be with small sample size (Aickin, 2007). However, the follow-up studies with the analysis of complete endocrinology and detailed survey on changes in uterus and ovaries would add the therapeutic value to this novel extract.

5 | CONCLUSION

As in the case of postmenopausal women, somatic, psychological, and urogenital issues are prevalent among perimenopausal participants as well, though with varying degree of severity. In the current study, the supplementation of a standardized extract of fenugreek having a unique 3:1 ratio of protodioscin to trigonelline concentrations (FHE) at 250 mg × 2/day for 42 days contributed significant improvement in somatic, psychological and urogenital scores; particularly on vasomotor symptoms and depression. A significant reduction in severity of symptoms was evident on day 28. Further, intra-group comparison of the hormone levels revealed significant variations in serum concentrations of estradiol, free testosterone, progesterone, FSH, and SHBG toward achieving a hormonal balance. The observed changes in hormone levels were within the safe reference range indicating the safety of FHE. Further hematological and biochemical analysis also demonstrated the absence of any toxic changes with FHE.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

Aman Khanna: Investigation; Supervision. **Febi John:** Formal analysis; Writing-original draft; Writing-review & editing. **Syam Das:** Formal analysis; Software; Writing-review & editing. **Jestin Thomas:** Data curation; Formal analysis; Methodology; Project administration; Software; Validation; Writing-review & editing. **Jyoti Rao:** Methodology; Project administration; Writing-original draft; Writing-review & editing. **Balu Makiakel:** Conceptualization; Funding acquisition; Resources. **Krishnakumar IM:** Conceptualization; Funding acquisition; Visualization; Writing-review & editing.

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