Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Safety and influence of a novel extract of fenugreek on healthy young women: a randomized, double-blinded, placebo-controlled study

Aman Khanna[1], Jestin Thomas[2], Febi John[3], Balu Maliakel[3] and I. M. Krishnakumar[3*]

Abstract

Background: Fenugreek (*Trigonella foenum-graecum*) seed is a popular kitchen spice and medicinal herb with wide applications in Indian folklore. Earlier studies have shown that the hydro-ethanolic extracts of fenugreek are efficient in the management of a number of hormone related disorders in women, including post and peri-menopausal discomforts, sexual dysfunctions, lactation and even in amenorrhea. However, systematic informations on their safety and influence on hormonal balance are limited.

Results: Forty-eight healthy menstruating women aged 20 to 48 were randomized either to FHE ($n = 24$) or placebo ($n = 24$) and supplemented with 250 mg × 2/day for 42 days. FHE did not produce any side effects or adverse events. It offered significant ($P < 0.05$) beneficial effects to sexual problems (41.6%) and irritability (40%) among the participants who had higher sexual dysfunctions scores (> 1) when monitored by the validated Menopausal Rating Scale (MRS) scale. Further, hormone analysis indicated an enhancement in estradiol ($P = 0.040$), free testosterone ($P = 0.025$), and total testosterone ($P = 0.012$) in FHE group in comparison to placebo. There were no significant changes in progesterone ($P = 0.174$) and FSH ($P = 0.879$) upon FHE supplementation. The hematological and biochemical safety parameters were also at par with the safety of the extract.

Conclusion: Thus, the supplementation of FHE may be considered as a natural alternative for sexual issues in women.

Trial registration: CTRI/2018/09/015614 dated 05/09/2018.

Keywords: Fenugreek, Irritability, Menopausal rating scale, Sexual problems, *Trigonella foenum-graecum*, Women

Introduction

The reproductive cycle is regulated by the hormones of the hypothalamic-pituitary-gonadal (HPG) axis; mainly the estradiol, progesterone, testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) [1]. Puberty starts with the rise in LH and estradiol secretions followed by the first menstruation [2]. In the reproductive years, cyclic rise of estrogen and progesterone occurs. While increase in estrogen and progesterone promoted endometrial proliferation and ovulation, the decreased level leads to endometrial mucosal degeneration (i.e., menstruation) [3]. Estradiol and progesterone levels start declining with the perimenopausal stage and return to pre-adolescent levels by the menopause, which usually occur between the age of 45 and 55 [3]. LH and FSH increase in the absence of ovarian estrogenic feedback [2]. Typical variations in the hormone levels during the life cycle of a woman can be depicted as Fig. 1 [4].

The constant variations in the hormonal levels normally cause a number of physiological and psychological changes in a woman's life. During the menstrual phase, woman may experience mood swings and Dysmenorrhea,

* Correspondence: krishnakumar.im@akay-group.com
[3]R&D Center , Akay Natural Ingredients Ltd., Cochin, India
Full list of author information is available at the end of the article

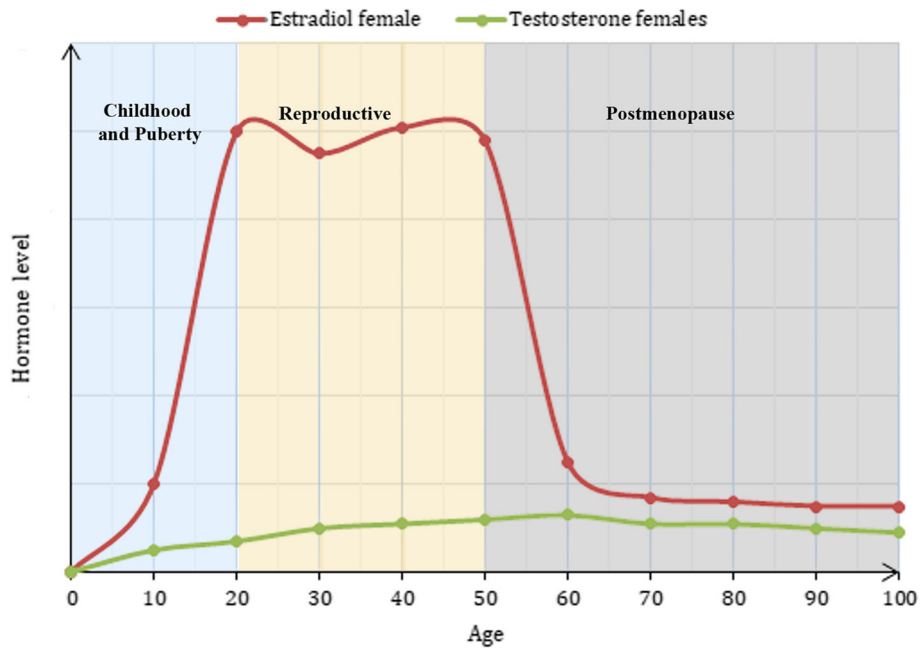

Fig. 1 Variations in hormone levels during the lifetime of women

a severe lower abdominal pain of cramping nature accompanied by vomiting, headache, back pain, diarrhoea and fatigue [5]. Prostaglandins produced by the linings of the uterus have been identified as one of the reasons for the pain since it is known to cause abnormal contractile activity leading to ischemia and hypoxia of the uterus and hence increased sensitivity of the nerve endings [6]. Non-steroid anti-inflammatory drugs (NSAIDs) are recognized as the first-line treatment in dysmenorrhea [7], though 25 to 30% failure normally reported [8]. At the menopausal phase, women experience physiologic deterioration of hypothalamic-pituitary-ovarian axis function resulting in hormonal imbalance and a variety of symptoms such as hot flushes, sweating, sleep disturbance, mood swings, depression, cognitive decline, vaginal dryness and sexual dysfunction occur with a significant impact on their quality of life [9, 10].

Several herbal remedies have been reported for alleviating various issues associated with various stages of life of a woman, primarily due to the hormonal variations [11]. Fenugreek (*Trigonella foenum-graecum*) is a popular kitchen spice and medicinal herb that has been shown to possess many useful properties against various physiological issues of women. Fenugreek seeds have been reported to alleviate dysmenorrhea associated with menstruation [12]; supports breast size due to the mastogenic effect [13]; promotes healthy sexual life and mood with a significant impact on libido and vitality among young women [14]; helps to reduce inflammation and body pain associated with child birth [12]; helps the contraction of uterus after delivery and is a galactogogue [15]. In the case of menopausal women, it has been shown to ameliorate various menopausal discomforts related to vasomotor, urogenetic, psychological issues [16, 17]. Despite all the benefits, human safety studies especially looking at the hormonal safety of fenugreek and its extracts are limited.

Recently, a hydro-ethanolic extract of fenugreek seeds (FHE; Patented and registered as FenuSMART®) containing protodioscin and trigonelline in a 3:1 ratio with around 10% of protodioscin was reported to alleviate both post and peri-menopausal discomforts when supplemented at 250 mg × 2/day for 42 days [16, 17]. These studies also have revealed a significant hormonal change (estradiol, progesterone, and testosterone) towards attaining a normal balanced hormone levels among menopausal women [16, 17]. FHE was shown to be tolerated upon supplementation at 500 mg × 2/day for 90 days on 88 postmenopausal women, without significant side effects or adverse events [18]. Therefore, we hypothesised that FHE would also be safe on healthy menstruating women since none of the early studies have shown uncontrolled variations in the reproductive hormones when supplemented to post and peri-menopausal women. Thus, the objective of the present study was to investigate the safety and influence of FHE on the hormone levels of young women at 250 mg × 2/day for 42 days.

Materials and methods

Recruitment of study participants

A total of 48 healthy menstruating women with regular menstrual cycles of 28–34 days and aged 20 to 48 years

were enrolled for the study. Study participants were identified and recruited from those who accompanied the patients at the outpatient's section of M/s Aman Hospital and Research Centre, Gujarat, India. The study was organized and conducted (generated the random allocation sequence, enrolled and assigned participants to intervention) under the supervision of a qualified Gynaecologist and Nutritionist with the help of a clinical research organization. Participants with MRS (Menopause Rating Scale) score greater than one for either sexual problem or irritability were selected for the study. Detailed inclusion and exclusion criteria are listed in Table 1. A medical screening comprising anthropometric measurements was conducted with all participants.

Intervention

The proprietary hydro-ethanolic extract of fenugreek seeds (FHE) having a 3:1 (w/w) protodioscin to trigonelline (Patented & Registered as 'FenuSMART®) manufactured in their good manufacturing procedure (GMP)-certified plant was obtained from Akay Natural Ingredients, Cochin, India along with a detailed certificate of analysis. High performance liquid chromatography (HPLC) analysis reported that FHE consist of protodioscin (10.3%), trigonelline (3.1%), 4-hydroxyisoleucine (2.8%). Food grade microcrystalline cellulose was employed as the placebo. The quality requirements, mainly related to safety such as microbial content, mycotoxins, heavy metals and pesticides for both FHE and placebo were analysed and certified by an accredited laboratory following the guidelines US pharmacopeia for dietary supplements [19]. FHE and placebo were identically packed into hard-shell, two-piece gelatin capsules, each containing 250 mg of FHE or placebo. The lethal dose (LD_{50}), acute (14 days) and repeated dose subchronic (90 days) toxicity of FHE have already been assessed [20].

Table 1 Inclusion and exclusion criteria

Inclusion criteria
1. Age 20–48 years (both inclusive).
2. Participants having BMI ≤ 30.
3. Participants with normal blood pressure and fasting blood glucose.
4. Participants understand the study procedures and provides signed informed consent to participate in the study.
Exclusion criteria
1. History of cerebrovascular disease, heart attack, or angina at any time or on anti-coagulant or anti-platelet drugs on a daily basis for any conditions.
2. Any previous hormonal treatment or treatment with fenugreek derived products in the previous 12 months.
3. Participants with abnormal ECG, biochemical or hematological values.
4. Any history of using estrogen or progestin containing products in the past 6 months of recruitment.
5. History of breast, endometrial, other gynecological cancer at any period of life or other cancer within the last 5 years.
6. Experiencing depression and/or receiving medication for such illness or disorders, receiving statins or other drugs known to impact on steroid hormone levels.
7. Participants, who are smokers, tobacco or alcohol user.
8. Participated in a clinical study with an investigational drug or biologic within the last 30 days.
9. Use of any recreational drugs (cocaine, amphetamine, barbiturates, benzodiazepines, cannabinoids and morphine)
10. History of clinically significant illness or any other medical disorder that may interfere with treatment, assessment or compliance with the protocol.
11. Any condition that in opinion of the Investigator, does not justify the participants participation in the study.

Study design

The present study was designed and conducted in a randomized, double-blinded, placebo-controlled and parallel group design. The study was in accordance with the clinical research guidelines of Government of India following the protocol evaluated and was registered in the clinical trial registry of India at http:/ctri.nic.in (CTRI/2018/09/015614 dated 05/09/2018).

The details of the experimental procedures including nature and risk were informed to all participants and their written informed consent were obtained before the study. A total of 48 menstruating women were selected by purposive sampling. Baseline sociodemographic variables such as age, race, civil status, marital status, and education were also noted. Participants were randomized to two intervention arms to receive either FHE (n = 24) or placebo (n = 24). Capsules containing 250 mg of either FHE or placebo were added to sequentially numbered airtight containers and provided on visit 1 (day 0). The participants were instructed to take two capsules per day (250 mg × 2) (one after breakfast and other after dinner) for 42 days.

A Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study is shown in Fig. 2a. The participants were monitored through regular telephonic follow-ups and short message services on a weekly basis, through which the daily drug administrations and the details of side effects or discomforts (if any), were enquired. Details of the analyses performed during each visits of the study period were as shown in Fig. 2b.

Randomization and blinding

Participants were randomly assigned into two groups using the random allocation sequence. The contract research organization staff who was not involved in the screening of participants handled the randomization sequence schedule. The study coordinator who maintained the allocation sequence was updated with the treatment

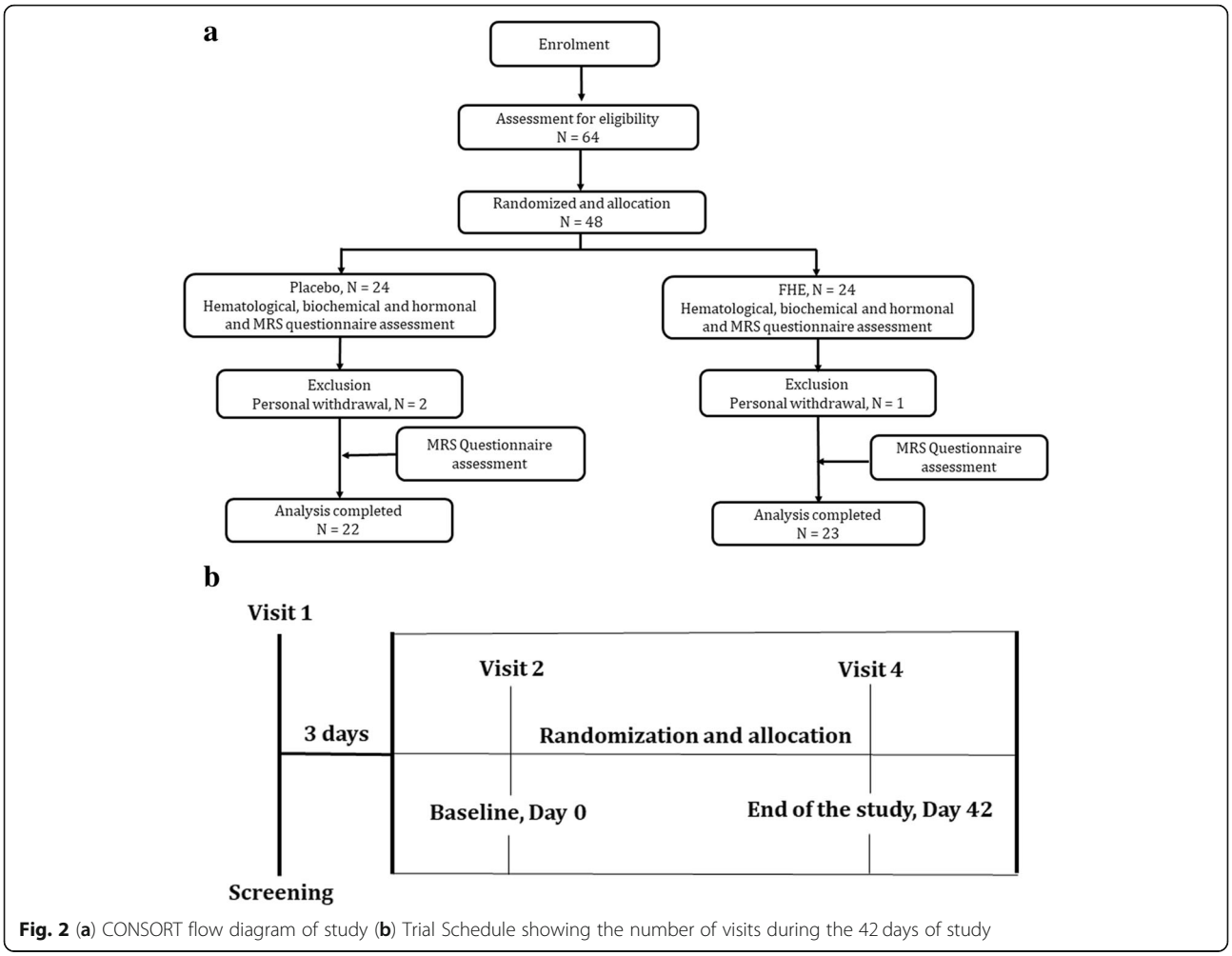

Fig. 2 (**a**) CONSORT flow diagram of study (**b**) Trial Schedule showing the number of visits during the 42 days of study

assignment, while the participants and the investigator were blinded to the intervention group.

Outcome measures

The primary outcome included the efficacy evaluation of supplementation of FHE on sexual problems and irritability as measured by menopause rating scale (MRS) questionnaire on day 0 and day 42. MRS is a health-related quality of life monitoring scale developed to measure the severity of menopausal symptoms, including sexual problems and irritability. The questionnaire consists of 11 items, self-completed by the participants at baseline (day 0) and end of study (day 42). The symptoms are classified as somatic (questions 1 to 3 and 11), psychological (questions 4 to 7) and urogenital factor (questions 8 to 10). The severity of complaints of each item is described on a 5-point rating scale (score of zero-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 [21].

The secondary outcome included the safety and influence of FHE on hormonal balance and comparing it with placebo. Both baseline (Day 0) and end of study (day 42) blood samples were collected by vein puncture and taken into EDTA/non-EDTA vials for hematological and biochemical parameters assay. Serum was separated by centrifugation at 6000 rpm at 4 °C for 10 min and stored at − 80 °C for further analysis. The hormones were analysed in serum samples using ELISA kit methods. Human estradiol ELISA kit (Biocompare, USA), progesterone ELISA (Cayman Chemicals, USA), follicle stimulating hormone (FSH) (Abcam, UK), free and total testosterone ELISA kits (Abcam, UK) were employed for analysis. The haematological parameters were assessed using an autoanalyzer (Meril Biochemistry analyzer, Eris diagnostics, and Instruments, India) and biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lipid profiling (total cholesterol, HDL cholesterol, LDL cholesterol,

VLDL cholesterol and triglycerides), serum creatinine and fasting blood sugar (FBS) were analysed with assay kits provided by M/s Agappe Diagnostics Pvt. Ltd. Bangalore, India.

Statistical evaluation

The primary efficacy evaluation included the MRS questionnaire based assessment scores corresponding to each of the discomforts at baseline and end of study (day 42). Secondary outcomes including hormonal markers and routine haematological and biochemical markers of safety were assessed at baseline and end of the study period (day 42).

Statistical analyses were carried out using the statistical Package (SPSS Inc. Chicago, IL, USA) version 25.0. Independent *t*-test was used for group comparison (FHE group verses control group) of demographic baseline characteristics and MRS scores. For evaluating the potential changes in hormone levels, biochemical and hematological parameters between placebo and FHE supplemented groups, a 2×2 repeated measures (RM) analysis of variance were performed with Bonferroni post hoc corrections. The results were presented as mean ± SD and $P \leq 0.05$ was considered significant.

Results

Study participants

Out of the forty-eight participants enrolled, 44 participants successfully completed the study. Three participants were (two from placebo and one from FHE group) withdrawn from the study due to personal reasons. Twenty-two participants in placebo and 23 participants in FHE completed the study as depicted in the cohort diagram in Fig. 2a & b.

Baseline characteristics

The baseline demographic characteristics measured were statistically insignificant ($P > 0.05$). The participant's sociodemographic variables are presented in Table 2. The mean age of study participants was 42.85 ± 4.2 and 41.10 ± 3.7 years, respectively for placebo and FHE groups. The mean baseline body weight of placebo and FHE were 62.3 ± 4.2 and 64.8 ± 4.5 respectively, baseline BMI in placebo was 23.7 ± 1.5, and that in FHE was 24.2 ± 1.85. The total, somatic, psychological and urogenital baseline scores as per MRS were 2.26 ± 1.28, 0.47 ± 0.73, 0.87 ± 0.81, 0.91 ± 0.84 in FHE group and 2.27 ± 1.27, 0.45 ± 0.59, 0.86 ± 0.64 and 0.95 ± 0.84 in placebo, respectively.

Table 2 Baseline demographic characteristics of study participants

Characteristics	Placebo ($n = 22$)	FHE ($n = 23$)
Age (years)	42.85 ± 4.2	41.10 ± 3.7
Weight (kg)	62.3 ± 4.2	64.8 ± 4.5
BMI (kg/m^2)	23.7 ± 1.5	24.2 ± 1.85
Education	n,%	n,%
Below high school	6 (25)	5 (22.7)
High school and above	16 (75)	18 (77.2)
Occupation		
Employed	17 (85)	16 (72.7)
Home-maker	5 (10)	4 (13.6)
Marital status		
Married	15 (68)	14 (61)
Divorced	1 (5)	3 (13)
Widow	6 (27)	6 (26)
Residence		
Urban	16 (72)	19 (83)
Rural	6 (27)	4 (17)
Exercise		
Never	12 (54)	14 (61)
Often	4 (18)	5 (22)
Daily	5 (23)	4 (17)
MRS		
Total score	2.27 ± 1.27	2.26 ± 1.28
Psychosocial score	0.86 ± 0.64	0.87 ± 0.81
Somatic score	0.45 ± 0.59	0.47 ± 0.73
Urogenital score	0.95 ± 0.84	0.91 ± 0.84

BMI- Body mass index, MRS- Menopause rating scale. Values are expressed as mean ± SD

Influence of FHE as monitored by MRS questionnaire

Deviations in MRS scores were primarily used for evaluating the influence of supplementation at baseline (day 0) and day 42. There was a significant ($P \leq 0.05$) improvement in the total MRS scores among FHE group (40.38%) as compared to placebo (16%) during the 42 days of study period. Somatic, psychological and urogenital symptoms scores showed 36.3, 35.0, and 47.6% reduction in FHE group, while the placebo showed 10.0, 15.7, and 33.3% improvements by the end of the study period. Further analysis of the individual symptom scores showed a significant reduction in the sexual problems (41.67%) in FHE group when compared to placebo (18.18%). The irritability scores were decreased by 40% in FHE group and 20.2% in placebo as shown in Fig. 3.

Effect of FHE on hormone balance

There was a significant increase in the levels of hormones after FHE supplementation. An increase in estradiol ($P = 0.04$), total testosterones ($P = 0.012$), and free testosterone ($P = 0.025$) were observed in the FHE group compared to placebo (Fig. 4). There were non-significant

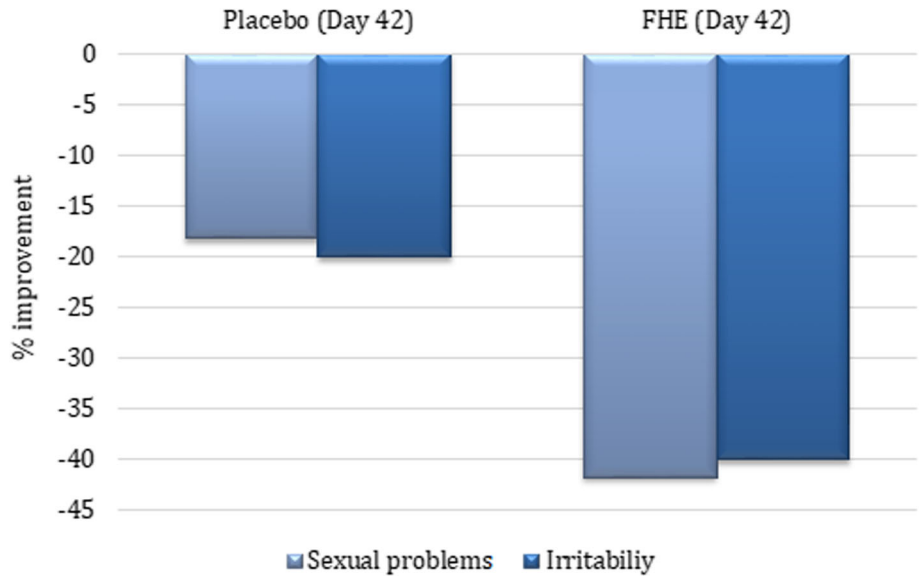

Fig. 3 Percentage improvement in sexual problems and irritability

changes in the levels of progesterone ($P = 0.174$) and FSH ($P = 0.879$) in FHE group when compared to placebo and the intergroup comparison also revealed no significant changes (Table 3). Individual analysis of hormone concentrations revealed that only 18 participants out of 24 in the FHE group had showed an increase in estradiol level. The average percentage increase was 21%, in which only seven participants showed above 10% increase in estradiol. In the case of testosterone, the average increase in total testosterone levels was 20%. The increase was below 10% among participants who reported total testosterone value above 30 ng/dL. Similarly, free testosterone levels showed an average of 24% increase, but the participants with free testosterone levels greater than 30 pg/dL had less than 10% increase (data not shown).

Safety and tolerance of FHE supplementation

During the study period, FHE was well tolerated without any adverse events. The assessment of hematological and biochemical parameters showed no significant changes in the baseline values (Table 4).

Discussion

Hormonal changes occur as women ages through menstrual cycles and menopausal phases as depicted in Fig. 1. The decline in androgen levels normally starts long before menopause; specifically from the perimenopausal age of around 47.5 years [22–24]. By the mid-30s or 40s, the normal activities of ovaries reduce and the process of ovulation becomes irregular [25]. In the perimenopause period, the levels of estradiol and progesterone decreases and in menopause, ovary can no longer release ova, and LH as well as FSH increase in the absence of ovarian estrogen feedback [2]. Estradiol and progesterone modulate sexual desire in women and the gradual and age-related cessation of ovarian function associated with natural menopause decreases the levels of these steroidal hormones and hence contribute towards the diminished sexual desire. This is a general observation among a significant portion of postmenopausal women [26].

The present study following a purposive sampling plan to select healthy women having some degree of sexual issues and irritability problems as reflected in the MRS scores, demonstrated a significant increase in estradiol and testosterone levels with a significant reduction in sexual issues and irritability scores when supplemented with FHE for 42 days at 250 mg × 2/day. About 81% of participants in placebo and 88% in FHE group reported scores between one and four for sexual problems and irritability, indicating mild to medium issues with regard to sexual functioning. However, the participants did not report to have any notable vasomotor symptoms at the time of enrolment. Upon hormonal analysis, it was found that these women had relatively low levels of estrogen and testosterone though within the reference range.

Estradiol and testosterone are the major hormones that have been implicated for modulating women's sexual desire and functions [26]. These hormones activate the Hypothalamic-Pituitary-Gonadal (HPG) axis by binding with estrogen receptors and the activation of HPG axis is necessary for fertility [27, 28]. Decrease in serum estrogen causes a decrease in clitoral intracavernosal, vaginal, and urethral blood flow, due to diffuse

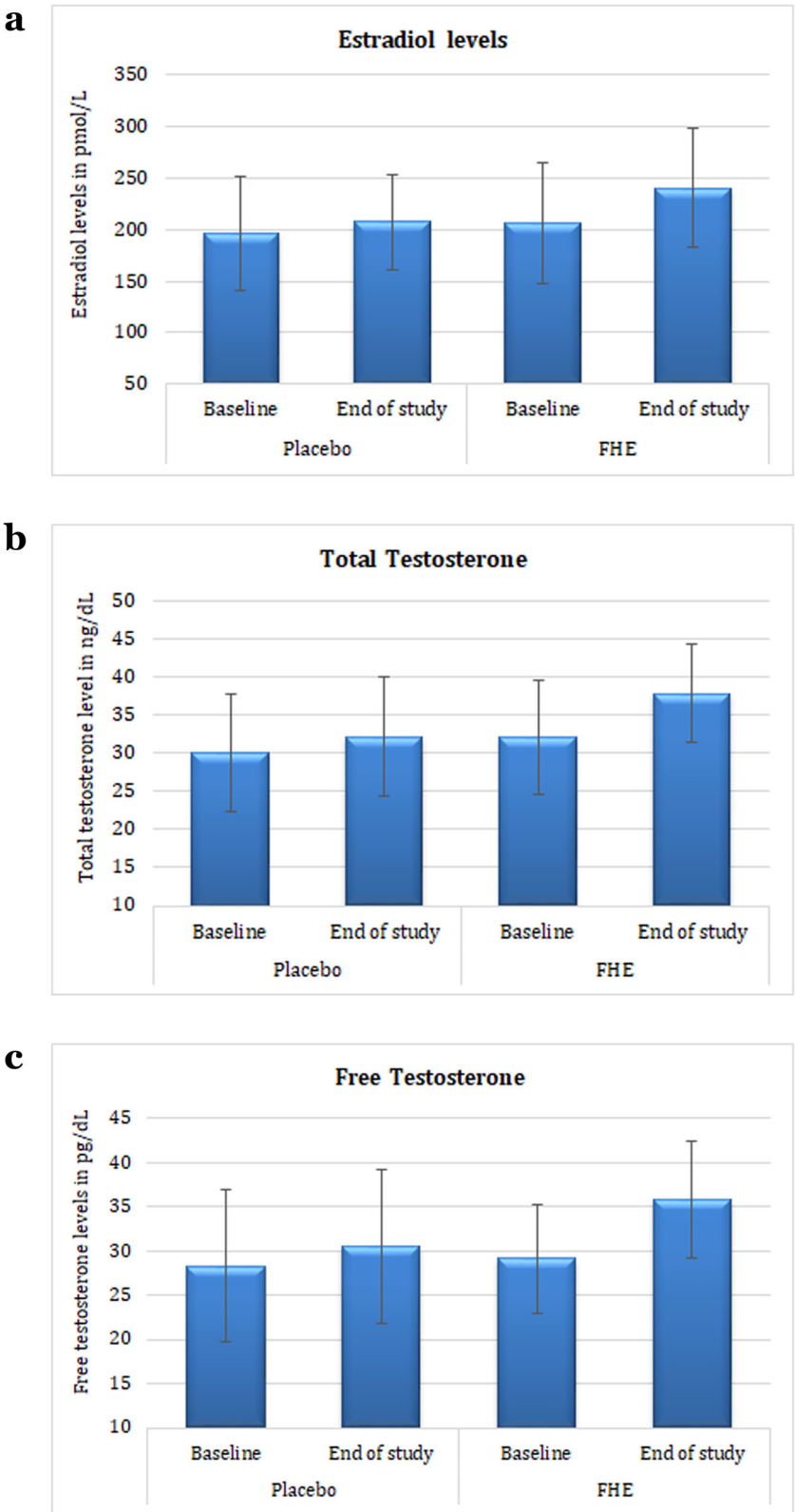

Fig. 4 Changes in the levels of (**a**) Estradiol (**b**) Total testosterone and (**c**) Free testosterone in FHE and placebo group at baseline and day 42

Table 3 Hormone levels of placebo and FHE groups

Parameter	Placebo		FHE supplemented group		*P*-value
	Baseline	End of Study	Baseline	End of Study	
Estradiol (pmol/L)	196.8 ± 55.4	207.1 ± 45.9	206.7 ± 58.4	240.3 ± 57.9[#]	0.040
Progesterone (nmol/L)	2.47 ± 0.92	2.59 ± 0.82	2.61 ± 0.70	2.9 ± 0.58	0.174
Follicle stimulating hormone (IU/L)	13.86 ± 4.54	13.15 ± 4.47	14.36 ± 5.69	12.97 ± 5.89	0.879
Total testosterone (ng/dL)	30.02 ± 7.65	32.14 ± 7.79	31.94 ± 7.47	37.75 ± 9.29[#]	0.012
Free testosterone (pg/dL)	28.25 ± 8.59	30.49 ± 8.72	29.11 ± 6.09	35.8 ± 6.7[#]	0.025

Hormone levels of placebo and FHE groups at baseline vs end of study. A 2 × 2 repeated measures (RM) analysis of variance were performed with Bonferroni post hoc corrections. Values are expressed as Mean ± SD. [#] indicate baseline vs end of study at $P \leq 0.05$

clitoral brosis, thinned vaginal epithelial layers, and decreased vaginal submucosal vasculature [29, 30]. In our study, the level of estradiol was significantly increased in FHE group and no significant change was observed in placebo group at the end of the study (Table 3). This has further correlated with the results of MRS scores, especially in the percentage improvement in sexual problems and irritability scores (Fig. 3). There was no significant change in the hormone levels or MRS scores in placebo.

The observation that the supplementation of FHE increased the estrogen and testosterone levels and significantly improved the quality of sexual functions and quality of life among participants who had issues at the baseline levels indicates the beneficial effect of FHE [31]. Earlier studies have also reported significant enhancement in both estradiol and testosterones among menopausal and healthy population having low libido when supplemented with fenugreek extracts [14, 16–18]. Elevated concentrations of endogenous estrogen and hormonal therapy with estrogen have already been reported to ease the menopausal discomforts [32]. However, the enhancement in estradiol and testosterone among participants with normal baseline levels were not significant ($P > 0.05$), indicating relatively weak estrogenic activity for FHE. Phytoestrogens are generally shown to possess weak binding with receptors [33]. So, under normal healthy conditions when there is enough natural estrogen in the body, the receptors available for binding with phytoestrogens will be limited. But, in the case of insufficient estrogen levels, phytoestrogens may bind to the receptors and support to ameliorate the symptoms associated with low estrogen levels. Phytoestrogens can also affect estrogen levels in ovarian tissue by interfering with estrogen-synthesizing and metabolic enzymes such as aromatase and estrogen receptors signalling pathways [34].

Androgens play an important role in healthy female sexual function, especially in stimulating sexual interest and maintaining desire [35]. Testosterone initiates sexual activities and proliferates sexual desire and behaviour. In addition, testosterone is essential in modulating clitoral and vaginal physiology to facilitate genital lubrication, sensation, and engorgement [36]. The greatest decline in circulating testosterone occurs during the late reproductive years [37]. Lack of testosterone has been reported to contribute to low libido and reduced sexual pleasure characterised by low sexual motivation, fatigue, distress, and overall reduction in the sense of well-being [33–35].

Oral and transdermal testosterone delivery has been shown to improve self-reported sexual satisfaction among premenopausal and postmenopausal women with low libido [36, 37]. A recent report has also evidenced the use of testosterone therapy to improve components of female sexual dysfunction [38]. Thus, the baseline sexual issues reflected by the MRS score in the present study can be dedicated to the lower normal range of testosterone. The increased total testosterone and free testosterone level after FHE supplementation might have also resulted in the improved sexual functions and libido.

Testosterone levels decline with age prior to menopause [39, 40]. There is ~ 50% fall in both total and free testosterones between the ages of 20 and 40–45, with only a very slight fall in circulating concentrations thereafter (Fig. 1). In a study on 149 healthy premenopausal women having no low libido complains and regular cycles, it was shown that a statistically significant decline for each of free testosterone, dehydroepiandrosterone sulfate (DHEAS), androstenedione and Dihydrotestosterone (DHT) occur with aging [41]. Testosterone can be converted to either DHT or estradiol by the enzymes 5α-reductase and aromatase, respectively [42]. Dehydroepiandrosterone (DHEA) is the principal precursor of both androstenedione and testosterone androgens and 50% of DHEA is produced in the adrenal glands and 20% from the ovaries; 30% is derived from DHEAS that circulates in the blood [43]. During post-menopause, up to 60% of DHEA tend to decline, resulting in hypoandrogenism which can affect the normal sexual response in women [44].

The molecular mechanism behind the beneficial effect of FHE can be mainly attributed to its protodioscin content, a furostanolic saponin molecule having a significant structural similarity with DHEA. It was shown that protodioscin can be converted to DHEA [45], which further

Table 4 Hematological and biochemical parameters in FHE and placebo

Parameters	Groups	Baseline	End of Study	*P*-value
Hemoglobin (g/dL)	Placebo FHE	15.3 ± 0.3 14.1 ± 0.4	14.8 ± 0.3 14.5 ± 0.4	0.075
Total leukocyte count (cells/cumm)	Placebo FHE	6072 ± 304.5 6195 ± 318.1	6178 ± 321.5 6305 ± 345.2	0.549
Total RBC count (million/cumm)	Placebo FHE	4.3 ± 0.12 4.5 ± 0.14	4.6 ± 0.11 4.4 ± 0.10	0.788
PC (lakhs/cumm)	Placebo FHE	2.5 ± 0.32 2.4 ± 0.3	2.3 ± 0.28 2.2 ± 0.25	0.325
PCV (%)	Placebo FHE	38.5 ± 1.5 34.3 ± 1.2	35.4 ± 1.1 32.3 ± 1.2	0.333
MCH (pg)	Placebo FHE	30.7 ± 1.4 32.3 ± 1.3	32.9 ± 1.5 31.8 ± 1.3	0.385
MCHC (g/dL)	Placebo FHE	85.5 ± 2.7 87.2 ± 2.5	82.1 ± 2.3 85.3 ± 2.4	0.323
MCV (fL)	Placebo FHE	92.7 ± 2.5 94.8 ± 2.8	95.1 ± 2.3 96.4 ± 2.5	0.961
Neutrophils (%)	Placebo FHE	52.7 ± 3.1 50.8 ± 3.0	55.1 ± 4.0 52.4 ± 3.7	0.439
Lymphocytes (%)	Placebo FHE	40.7 ± 2.0 42.2 ± 2.1	38.4 ± 2.2 36.9 ± 2.0	0.662
Eosinophil's (%)	Placebo FHE	4.2 ± 0.65 4.0 ± 0.72	4.1 ± 0.62 3.8 ± 0.55	0.795
Monocytes (%)	Placebo FHE	3.2 ± 0.65 3.1 ± 0.62	3.5 ± 0.52 3.3 ± 0.54	0.980
ESR (mm/hr)	Placebo FHE	10.4 ± 1.0 10.6 ± 1.2	8.4 ± 0.92 8.8 ± 1.1	0.342
Triglycerides	Placebo FHE	126.3 ± 4.5 129.5 ± 4.8	136.2 ± 5.2 132.5 ± 5.4	0.773
Total Cholesterol	Placebo FHE	192.4 ± 7.5 189.7 ± 7.2	185.7 ± 6.5 182.2 ± 6.2	0.642
HDL Cholesterol	Placebo FHE	40.2 ± 2.2 43.5 ± 2.5	42.7 ± 2.5 45.5 ± 2.5	0.698
LDL Cholesterol	Placebo FHE	152.8 ± 4.2 148.7 ± 4.0	158.2 ± 4.2 152.7 ± 4.8	0.380
VLDL Cholesterol	Placebo FHE	28.7 ± 7.2 26.1 ± 8.1	25.7 ± 6.8 27.2 ± 7.7	0.298
SGOT (U/L)	Placebo FHE	23.1 ± 4.2 26.7 ± 4.8	25.4 ± 4.9 27.3 ± 5.2	0.822
SGPT (U/L)	Placebo FHE	35.2 ± 7.2 38.5 ± 7.6	32.7 ± 6.7 35.3 ± 6.5	0.717
Alkaline phosphatase	Placebo FHE	55.7 ± 7.0 62.8 ± 7.8	52.7 ± 7.2 55.3 ± 7.4	0.353

RBC- Red blood cells, PC- Platelet count, PCV- Packed cell volume, MCH- Mean corpuscular hemoglobin, MCHC- Mean corpuscular hemoglobin concentration, MCV- Mean corpuscular volume, ESR- Erythrocyte sedimentation rate, HDL- High-density lipoprotein, LDL- Low-density lipoprotein, VLDL- Very low-density lipoprotein, SGOT- Serum glutamate oxaloacetate transferase, SGPT- Serum glutamate pyruvate transferase. 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. $P \leq 0.05$ considered as statistically significant with baseline

gets converted to testosterone and estradiol. Aromatase is involved in the conversion of testosterone to estradiol [46]. Zhou et al. have reported a significant increase of *17β-Hydroxy Steroid Dehydrogenase (17β-HSD), 3β- Hydroxy Steroid Dehydrogenase (3β-HSD)* and *aromatase* mRNA levels when female rats were treated with DHEA [47]. The enzymes *17β-HSD* and *3β-HSD* are important in the synthesis of steroid hormones such as testosterone and estradiol which are involved to activate the Hypothalamic-Pituitary-Gonadal (HPG) axis by binding with estrogen receptors [27, 28]. Trigonelline, yet another major component in FHE has also been proven to enhance the estrogen level by some unknown mechanisms [48].

In women, abnormally high concentration of testosterone has been associated with polycystic ovary syndrome (PCOS) and ovarian cancers [49]. Serum levels of testosterone has been suspected in the etiology of breast cancer of postmenopausal women [50]. Most testosterone values in PCOS will be ≤150 ng/dL (≤ 5.2 nmol/L) [49], and in ovarian or adrenal tumour, it is shown to be of ≥200 ng/dL (≥ 6.9 nmol/L). The enzyme like ovarian aromatase activity is lower under PCOS conditions [51]. This may limit the conversion of testosterone to estradiol as well. So, the consumption of fenugreek extracts by women having PCOS conditions have to be careful, though earlier study has reported the beneficial effects of fenugreek under PCOS condition [52]. However, the role of phytoestrogens is ambiguous and they bind weakly to estrogen receptors and can either produce or inhibit estrogen effects [53]. In the present study, no overproduction of hormones has been observed with FHE intake, probably due to the active feedback mechanism in healthy women, which warrants further study on healthy participants. Since, estradiol is known to increase the concentration of serotonin and modulate its action, the effects of FHE on serotonin pathways also can be investigated to check the beneficial effects of FHE in menopausal discomforts and sexual issues.

Conclusions

In summary, supplementation of FHE at 250 mg × 2 per day for 42 days did not produce any side effects, adverse events or significant changes in both the haematological and biochemical parameters in young menstruating women. Further analysis indicated no detrimental effect on hormonal balance. The enhancement observed for estradiol and testosterone concentrations were within the safe reference range. The molecular mechanism of estrogenic effect can be attributed to the protodioscin content in FHE, since protodioscin can be converted to DHEA and further to testosterone and estradiol by the action of aromatase. The present study showed that FHE helps to maintain normal hormonal balance and offered a significant reduction in sexual problems and irritability scores among the participants.

Abbreviations

HPG: Hypothalamic-pituitary-gonadal; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; NSAIDs: Non-steroid anti-inflammatory drugs; FHE: Fenugreek seed extract; MRS: Menopausal rating scale; LD_{50}: Lethal dose; CONSORT: Consolidated standards of reporting trials; CTRI: Clinical trail registry of India; EDTA: Ethylenediamine tetraacetic acid; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol; VLDL: Very low-density lipoprotein cholesterol; FBS: Fasting blood sugar; BMI: Body mass index; PCOS: Polycystic ovary syndrome; DHEAS: Dehydroepiandrosterone sulfate; DHT: Dihydrotestosterone; DHEA: Dehydroepiandrosterone; RBC: Red blood cells; PC: Platelet count; PCV: Packed cell volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; ESR: Erythrocyte sedimentation rate

Acknowledgements

The authors thank Thyrocare, Bangalore, India for providing the laboratory services.

Authors' contributions

Aman Khanna: Investigation; Supervision. Jestin Thomas: Data curation; Formal analysis; Methodology; Project administration; Software; Validation; Writing-review & editing. Febi John: Formal analysis; Writing-original draft; Writing-review & editing. Balu Makiakel: Conceptualization; funding acquisition; Resources. Krishnakumar IM: Conceptualization; funding acquisition; Visualization; Writing review & editing. The author(s) read and approved the final manuscript.

Funding

The study was financially supported by M/s Akay Natural Ingredients, Cochin, India as a part of the research program (Reg. No. AK/R&D/NPP-05/2018) for the development of '*Spiceuticals*®' – the nutraceutical ingredients from spices. The funders have developed the formulation, conceived the idea, and prepared the manuscript; but had no role in the execution of the study, sample collection, analysis and interpretation of data.

Availability of data and materials

The dataset analysed in the present study are available from the corresponding author on reasonable request.

Declaration

Ethics approval and consent to participate

The study was in accordance with the clinical research guidelines of Government of India following the protocol evaluated and was registered in the clinical trial registry of India at http:/ctri.nic.in (CTRI/2018/09/01 5614 dated 05/09/2018).

Consent for publication

Not applicable.

Competing interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. FHE used in the study is a patented extract of Akay Natural Ingredients, Cochin, India as FenuSMART®.

Author details

[1]Aman Hospital and Research Center, Vadodara, Gujarat, India. [2]Leads Clinical Research and Bio Services Pvt. Ltd., Bangalore, India. [3]R&D Center , Akay Natural Ingredients Ltd., Cochin, India.

Received: 19 February 2021 Accepted: 17 June 2021
Published online: 13 July 2021

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