



The effects of *Elaeagnus angustifolia* L. whole fruit on the sex hormone profile in menopausal women: A double-blind, randomized, placebo-controlled study



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ARTICLE INFO

Keywords:

Menopause
Elaeagnus angustifolia L.
Estradiol
Sex hormones
Traditional medicine
Iran

ABSTRACT

Ethnopharmacological relevance: Menopause is a product of interrupted ovarian activity and decrease in its estradiol production. Herbal medicines as an alternative to hormone therapy are increasingly used by menopausal women. *Elaeagnus angustifolia* L. (Senjed in Persian) is a well-known herbal remedy with various therapeutic effects according to Iranian traditional medicine which is recommended to relieve the menopausal side effects. The aim of present study was to evaluate the effects of oral intake of whole fruit powder of *E. angustifolia* on the sex hormones profile in menopausal women.

Materials and methods: In present double-blind randomized placebo-controlled trial, 58 eligible women who were referred to Kamali Women Hospital (Karaj, Iran, 2017) were randomly assigned into herbal medicine (15 g *E. angustifolia*) and placebo (7.5 g cornstarch + 7.5 g isomalt) groups. Initially and after 10 weeks of the treatment, serum levels of estradiol, progesterone, testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) hormones were measured.

Results: According to between-group analyses, the changes in the studied parameters were not significant between herbal medicine and placebo groups, except for joint pain that improved significantly in herbal medicine group. However, by within-group analysis the levels of FSH and FSH to testosterone showed a significant increase, whereas the level of progesterone decreased significantly after 10 weeks of *E. angustifolia* consumption.

Conclusions: The improvement of the sex hormone profile was not in a full accordance with Iranian folklore after *E. angustifolia* consumption in the present menopausal participants. However, considering a strong belief on the beneficial effects of *E. angustifolia* in Iranian folklore, a long-term studies of larger group participants are needed to evaluate the efficacy.

1. Introduction

Menopause is a condition that all women experience as they age (Navarro-pardo, Holland and Cano, 2018). Natural menopause occurs in women's lives when menstrual periods stop permanently and the function of ovarian follicleceases (Stuenkel et al., 2015). Hormonal changes during menopause, including decrease in the production of estradiol, and increase in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) lead to complex changes in the entire body of a

woman (Navarro-pardo et al., 2018; Yum and Kim, 2014). Menopause is associated with several symptoms that vasomotor symptoms like hot flashes are the most annoying ones (Stuenkel et al., 2015). Pathophysiology of vasomotor symptoms are still unknown, however, these symptoms are caused by the estradiol's discontinuation (Freedman, 2001) and is related to the secretion of the LH hormone from the pituitary and decrease in endorphin concentration in the hypothalamus (Polycove et al., 2011). During postmenopausal stage, ovary reduces estradiol and progesterone hormones and increases FSH, LH and

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<https://doi.org/10.1016/j.jep.2019.112229>

Received 19 December 2018; Received in revised form 18 August 2019; Accepted 8 September 2019

Available online 09 September 2019

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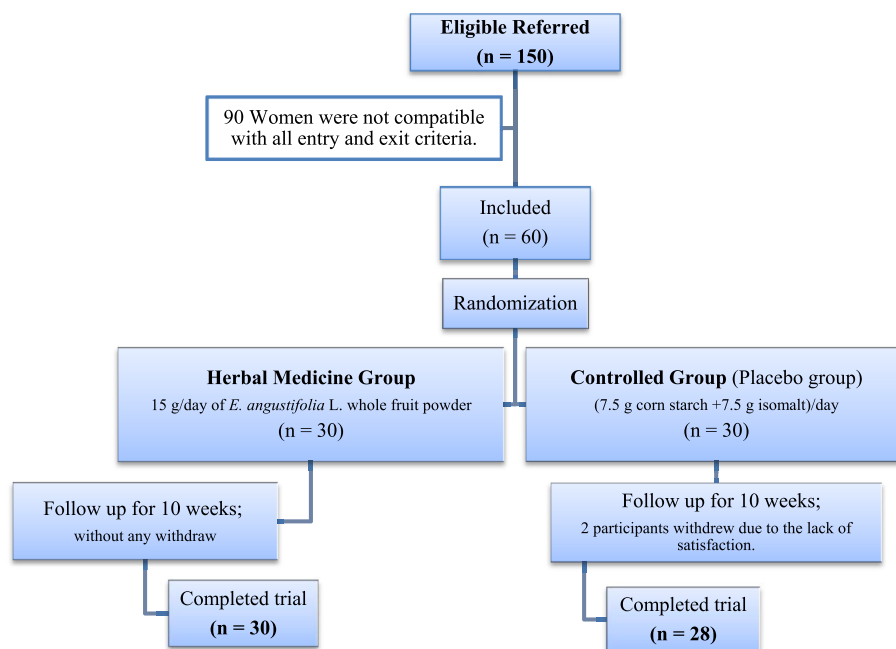


Fig. 1. Flow chart for patient enrollment, randomization, and retention.

testosterone hormones, due to feedback functions of the hypothalamic-pituitary axis hormones (Honour, 2018). Phytoestrogens have antioxidant activity and can be crossbred to estradiol receptors (Ososki and Kennelly, 2003). Since phytoestrogens can produce more potent estrogenic effects in the absence of estradiol, they can be used instead of estradiol and compensate the level of innate 17- β -estradiol reduction in menopause (Anandhi Senthilkumar et al., 2018).

Phytochemical studies of *Elaeagnus angustifolia* L. (based on www.theplantlist.org) extract indicate the presence of two phytosterols, including β -Sitosterol and its acetate form in fruit and fruit peel, and also stigmasterol in fruit peel (Reviewed by (Farzaei et al., 2015)). *E. angustifolia* contains various secondary metabolites, vitamins, minerals ingredients, amino acids, and carbohydrates. *E. angustifolia* fruit contains benzoic acid derivatives, vanillic acid, protocatechuic acid, p-coumaric acid and ferulic acid. Flavonoids mostly kaempferol, rutin, luteolin (Aman A et al., 2018; Reviewed by (Hamidpour et al., 2017; Tehranizadeh et al., 2016)) and quercetin are also identified in different parts of *E. angustifolia*. (Reviewed by (Farzaei et al., 2015; Tehranizadeh et al., 2016)).

E. angustifolia L. known as Senjed in Persian, which is belonged to the Elaeagnaceae family, is used as both a diet and a herbal medicine (Saboonchian et al., 2014). In several regions of Iranian rituals, *E. angustifolia* is a symbol for rationalism, wisdom and women's fertility (Matin, 2013). In Iranian traditional medicine, *E. angustifolia* was mentioned as costive agent, pain reliever, and anti-inflammation as well as anticonvulsants. Moreover, its ripe fruit is used to treat stomach disorders, amoebic diarrhea, and sexual function enhancement of female aphrodisiacs, liver and spleen revival (Farzaei et al., 2015; Güler et al., 2015; Nasab and Khosravi, 2014).

In folklore medicine of Iran, the use of *E. angustifolia* is recommended to relieve the menopausal side effects. The beneficial effects of *E. angustifolia* have been studied in cases such as female orgasmic disorders (Akbarzadeh et al., 2014), knee osteoarthritis (Ebrahimi et al., 2014; Rabiei et al., 2015) and symptomatic oral lichen planus (Taheri et al., 2010) in human, intragastric effect (Eliassi et al., 2008), antinociceptive, anti-inflammatory and muscle relaxant effects in rat (Ahmadiani et al., 2000; Tamtaji et al., 2014) and in mice (Hosseinzadeh et al., 2003; Karimi et al., 2010), as well its larvicidal activity against *Drosophila melanogaster* (Anjum et al., 2017). However, its effects have not yet been studied in menopausal women. This article

aims to investigate the effect of ripe *E. angustifolia* whole fruit on the sex hormones profile in postmenopausal women.

2. Material and methods

2.1. Study design

This double-blind placebo-controlled randomized trial was performed on the postmenopausal women who were referred to the gynecology clinic of the Kamali hospital. The study was registered in the clinical trial system with the registration code of IRCT2017030932795N2 and was approved by the relevant authorities with the ethical code of Abzums. Rec.1396.88. The purposes and method of the study were explained to eligible patients and an informed written consent was obtained from the participants. Kamali hospital is an academic center and the referral center for obstetrics and gynecology patients in Alborz province which is the fourth populous city of Iran (Karaj, Alborz, Iran).

2.2. Major inclusion and exclusion criteria for the study

Participants were invited according to the guidelines for inclusion and exclusion criteria to the study. Inclusion criteria included: 1) Menopause in the age range of 40–70 years; 2) The serum level of total cholesterol was between 200 and 300 mg/ml, with no need to medical treatment. Exclusion criteria included: 1) Cardiovascular and renal diseases; 2) Metabolic disorders, such as diabetes; 3) Those using psychiatric drugs; 4) Having destructive habits like smoking cigarettes and hookahs, consuming alcohol and drugs.

In addition to entry requirements, the women neither have a history of taking hormone injections in the last 6 months, nor a history of daily intake or sensitivity to *E. angustifolia*. In initial assessment, blood pressure, heart rate, total cholesterol, body mass index (BMI) and FSH, for the confirmation of being a menopause, were evaluated. Fig. 1 shows the flowchart of patient recruitment and retention.

2.3. Randomization and study groups

Using a simple randomization method, the experimental groups of the study included the herbal medicine group (full powdered *E.*

angustifolia) and the placebo group (isomalt powder and cornstarch in a 1:1 ratio) were chosen. There were 30 postmenopausal women in each group. The group of herbal medicine took 15 g of herbal medicine daily (ripe *E. angustifolia* whole fruit powder) and the placebo group took a combined mixture of 7.5 g of cornstarch and 7.5 g of isomalt for 10 weeks in parallel.

Dosages and duration of consumption of Senjed and placebo were chosen according to clinical trials on the effects of *E. angustifolia*, isomalt and corn starch on osteoarthritis (Ebrahimi et al., 2014), lipid profile and atherogenic profile in obese women (Nikniaz et al., 2016).

Also, based on the central limit theorem (CLT) (Kwak and Kim, 2017), the sample size is approximately 30. Therefore, the sample mean probability distribution of the studied characteristics is close to the normal probability distribution.

2.4. Preparation of treatments

E. angustifolia fruits were products of Damghan's gardens which were purchased in October 2017 from Ebrahimi Brothers store and Rezaei nut shop (Farhang square, Damghan). Then fruits were completely dried in Gharibi confectioner's shop (Business license 1273; Shahrood, Semnan, Iran) and were ground three rounds to change into a smooth powder after the identification and confirmation of the health and quality by Dr. Atefeh Amir Ahmadi (PhD of Plant Biosystematics, School of Biology, Damghan University) and Dr. Anna Abodolshahi (PhD of Food science and technology, Food safety research center (salt), Semnan, Iran). The specimen of *E. angustifolia* was kept in the herbarium of Damghan university (Voucher number, Amirahmadi: 1842 (DU000584)). Cornstarch from Bijan Pharmacy (Tehran, Iran) and Isomalt from PuyaKabak Company Manufacturing and Trading (Batch No1604112, Tehran, Iran) were prepared with food grade and combined in a 1:1 ratio. The herbal medicine and placebo packages were stored in a refrigerator for food before delivery to the participants and they were suggested to store their packages in refrigerator before consumption. Data collection was done by taking a fasting blood sample and filling in an interview questionnaire.

It was recommended to the sample of the study, to eat contents of the packages after breakfast and with milk, if possible (Nikniaz et al., 2016).

2.5. Sampling and laboratory analysis

Sampling, filling out a questionnaire and performing laboratory tests were done in Iranzamin Lab (Karaj) in two stages before treatment (in the beginning of the trial and before starting the herbal medicine/placebo) and after treatment (after 10 weeks of daily consuming of herbal medicine/placebo).

To measure the biological indices, a serum was prepared from 3 ml of patients fasting blood samples. Three estradiol, progesterone and testosterone hormones were measured by a fully automated Immulite 2000, Siemens, USA, with its own special kits and the chemoluminescence technique. FSH and LH hormones were measured by Enzyme-Linked Immunosorbent Assay (ELISA) and commercial Bio Karpira kits.

2.6. Statistical analysis

Between-group and within-group analyses of each studied parameter were performed for herbal medicine and placebo groups, before and after consumption using SPSS software (version 25). Between-group p-values show the significance of differences in the studied parameters between herbal medicine and placebo groups before and after the trial period while, within-group p-values show the significance of differences in the studied parameters of each study groups before and after the trial period. Change score p-values compare between p-values of each studied parameter.

In quantitative data, after determining whether our data were normal or not, they were analyzed with one of the following tests

including, *t*-test (between analysis of normal data), Pair *t*-test (within analysis of normal data), Mann-Whitney test (between analysis of abnormal data), and Wilcoxon test (within analysis of abnormal data). Moreover Chi-square test was used to examine the nominal data. We used change score analysis in order to compare the evidence of herbal medicine changes scores comparison. We considered a statistical significance of 0.05 ($p < 0.05$).

3. Results

In both herbal medicine (*E. angustifolia*) and placebo (isomalt and cornstarch) groups, women had a normal distribution based on age. The average age of the subjects (58 people) was 55.39 ± 6.26 years separated into the herbal medicine (30 people) and placebo groups (28 people) with the average age of 56.63 ± 5.43 and 54.07 ± 6.90 years, respectively. Differences between two experimental groups regarding this feature were not statistically significant ($p = 0.121$). In the placebo group, two people refused to continue cooperation.

Table 1 shows the usage methods of treatments and the abundance of menopause complications among herbal medicine and placebo groups. There were no significant differences between the two groups in the usage methods ($p = 0.478$) and in the different consumption times ($p = 0.869$). Around half of menopausal participants suffered from hot flushing, sleep disorders, fatigue, urinary urgent, anxiety and mood change. According to the questionnaire (Table 1), participants did not notice any changes after the course having *E. angustifolia* and placebo in complications. In between-group analysis, the distribution of menopausal complications were not significantly different between participants in both study groups, except for the urinary urgent, which was significantly higher in women who received the herbal medicine ($p = 0.014$). Contrary to the common belief in the folk culture of Iranian traditional medicine, *E. angustifolia* fruit powder did not cause constipation and digestive disorder in the women of this study. However, in the herbal medicine group, there was a high joint pain improvement and an average joint pain improvement in 50% and 20% of the participants, respectively, after taking the herbal medicine. The difference between the herbal medicine and placebo groups in changes of joint pain was statistically significant ($p = 0.0003$).

Table 2 shows the results for the effects of herbal medicine and placebo treatments on sex hormone profile. Due to the variation in the distribution and normality of data, the comparison of the effects was tested using different statistical methods. Considering within-group P-values, the amount of FSH shows a significant increase in both herbal medicine ($p = 0.020$) and placebo ($p = 0.042$) groups after ten weeks of treatment. However, by assessing the changes between-group, the change in comparative states of FSH level was not significant (change score p-value = 0.830) before and after consumption. In addition to FSH, the mean score of changes in estradiol and testosterone between two study groups were not significant (change score p-values = 0.772 and 0.696, respectively). As well, the increase in estradiol levels in the herbal medicine and placebo groups was not significant (within-group p-values = 0.108 and 0.066, respectively). Considering within-group p-values, changes in the level of testosterone were also not significant in herbal medicine and placebo groups ($p = 0.796$ and 0.382, respectively).

The decrease in LH levels was not significant in the herbal medicine and placebo groups (within-group p-values = 0.307 and 0.320, respectively). Whereas a significant decrease in progesterone levels was only seen in herbal medicine (within-group p-value = 0.049). The changes in LH (change score p-value = 0.933) and progesterone (change score p-value = 0.273) were not significant between the herbal medicine and placebo groups.

For hormone ratios, according to change score p-values, the increase in the ratios of estradiol to testosterone ($p = 0.488$), estradiol to progesterone ($p = 0.858$), FSH to estradiol ($p = 0.776$), FSH to progesterone ($p = 0.674$) and FSH to testosterone ($p = 0.919$) was not

Table 1

Questionnaires information on the age of the participants, as well the usage methods of herbal medicine (15 g of *E. angustifolia* whole fruit powder) and placebo (7.5 g cornstarch + 7.5 g isomalt) and the abundance of menopause complications before and after ten weeks of trial.

Variable	Category	Groups				Total (n = 58)	p-value		
		Herbal Medicine (n = 30)		Placebo (n = 28)					
Age	Numerical	56.63 ± 5.43		54.07 ± 6.90		55.39 ± 6.26	0.121 ^f		
Variables	Category	Total	Herbal Medicine		Placebo		Total		p-value
			No.	Percentage	No.	Percentage	No.	Percentage	
Hot flushing	Yes	1	15	50%	18	64.3%	33	56.9%	0.272*
	No	2	15	50%	10	35.7%	25	43.1%	
Night sweat	Yes	1	13	43.3%	13	46.4%	26	44.8%	0.813*
	No	2	17	56.7%	15	53.6%	32	55.2%	
Sleep disorders	Yes	1	16	53.3%	16	57.1%	32	55.2%	0.771*
	No	2	14	46.7%	12	42.9%	26	44.8%	
Fatigue	Yes	1	18	60%	19	67.9%	37	63.8%	0.534*
	No	2	12	40%	9	32.1%	21	36.2%	
Dysuria	Yes	1	9	30%	10	35%	19	32.8%	0.643*
	No	2	21	70%	18	64.3%	39	67.2%	
Urinary urgent	Yes	1	17	56.7%	7	25%	24	41.45%	0.014*
	No	2	13	43.3%	21	75%	34	58.6%	
Urinary incontinence	Yes	1	10	33.3%	4	14.3%	14	24.1%	0.090*
	No	2	20	66.7%	24	85.7%	44	75.9%	
Depression	Yes	1	12	40%	11	39.3%	23	39.7%	0.956*
	No	2	18	60%	17	60.7%	35	60.3%	
Anxiety	Yes	1	15	50%	16	57.1%	31	53.4%	0.586*
	No	2	15	50%	12	42.9%	27	46.6%	
Change in mood	Yes	1	13	43.3%	16	57.1%	29	50%	0.293*
	No	2	17	56.7%	12	42.9%	29	50%	
Dizziness	Yes	1	11	36.7%	12	42.9%	23	39.7%	0.630*
	No	2	19	63.3%	16	57.1%	35	60.3%	
Consumption times	Morning	1	18	60%	15	53.6%	33	56.9%	0.869 ^{tt}
	Noon	2	1	3.3%	0	0%	1	1.7%	
	Evening	3	1	3.3%	5	17.9%	6	10.3%	
	Night	4	2	6.7%	6	21.4%	8	13.8%	
	Various times	5	8	26.7%	2	7.1%	10	17.2%	
Method of use	Nothing	1	0	0%	1	3.6%	1	1.7%	0.478 ^{tt}
	Water	2	1	3.3%	8	28.6%	9	15.5%	
	Milk	3	9	66.7%	8	28.6%	28	48.3%	
	Honey	4	1	3.3%	0	0%	1	1.7%	
	Combine 4 top modes	5	8	26.7%	11	39.3%	19	32.8%	
Change in joint pain	Unchanged	1	5	16.7%	13	46.4%	18	31%	0.0003^{tt}
	Low improvement	2	4	13.3%	7	25%	11	19%	
	Average improvement	3	6	20%	6	21.4%	12	20.7%	
	High improvement	4	15	50%	2	7.1%	17	29.3%	

t: T-test; *: Chi-Square test, tt: Mann-Whitney test, No.: Number. Bold figures show that the difference is significant ($p < 0.05$).

significant. Considering within-group analyses of data, except for the ratio of estradiol to testosterone in placebo group with a p-value of 0.010 and in herbal medicine with a p-value of 0.067 (which can be considered significant with a significant level set at $p < 0.1$), the observed increase in studied hormone ratios was not significant.

4. Reasons for the lack of preclinical study

The current study was conducted without any preclinical studies due to following reasons.

Firstly, effects of *E. angustifolia* on lipid profile and atherogenic indices in obese females (Nikniaz et al., 2016), its effect on serum levels of inflammatory cytokines and matrix metalloproteinases in females with knee osteoarthritis (Nikniaz et al., 2014), comparison of its extract and sildenafil citrate on female orgasmic disorders (Akbarzadeh et al., 2014), its topical gel efficiency in the treatment of symptomatic oral lichen planus (Taheri et al., 2010) and its pollen allergenicity and cross-reactivity (Sastre et al., 2004) were previously studied in human. Except for a minimal-to-moderate cross-reactivity with olive pollen (Sastre et al., 2004) no adverse effects were reported for *E. angustifolia*.

Secondly, various effects of *E. angustifolia* were examined in animal models as follows. Healing effects on ulcerative colitis (Khodakarm-Tafti et al., 2015), effects of the leaf extract on lipidemic profile (Mirazi et al., 2015), cutaneous wound healing effects of the fruit's aqueous extract (Natanzi et al., 2012), anti-ulcerogenic activity (Gurbuz et al., 2003) and antinociceptive and anti-inflammatory effects of the fruit's aqueous extract (Ahmadiani et al., 2000) were studied in rats. Furthermore, inhibition of cyclooxygenase type 1 and 2 enzyme by its fruit aqueous extract (Farahbakhsh et al., 2011), its fruit seeds muscle relaxant activity (Hosseinzadeh et al., 2003) and antinociceptive effect of *E. angustifolia* fruit (Ramezani et al., 2001) were studied in mice. It is also noteworthy that no side effects were observed in above-mentioned animal studies. Although Talaei-Khozani et al. showed that water/alcohol extract of *E. angustifolia* fruit had some toxic effects on chondrogenesis and osteogenesis on in mouse embryo limb buds in vitro and in vivo (Talaei-Khozani et al., 2011). This finding might not be in contradiction to our study as the participants of the present study are menopausal women who used the whole fruit powder not its alcohol extract.

Thirdly, there are serious ethical and scientific criticism on the safety, reliability and the predictive value of data obtained from animal

Table 2

Profile of sex hormones and their proportions in the experimental groups before and after 10- week treatment of herbal medicine (15 g of *E. angustifolia* whole fruit powder) and placebo (7.5 g cornstarch + 7.5 g isomalt. Data are shown as mean \pm standard deviation.

Variables (units)	Time	Herbal Medicine Group (n = 30)		Placebo Group (n = 28)		Mean Difference	95% Confidence Interval of the Difference		p-value
		Mean \pm SD	Median	Mean \pm SD	Median		Lower	upper	
ⁿ FSH (IU/L)	Pre- treatment	40.94 \pm 21.58	36.81	39.78 \pm 22	31.64	1.611	-10.308	12.63	0.840 ^t
	Post-treatment	53.73 \pm 30.78	55.96	54.41 \pm 28.85	60.13	-0.680	-16.404	15.04	0.931 ^t
	Change score	12.78 \pm 28.36	10.74	14.63 \pm 36.32	10.15	-1.842	-18.923	15.238	0.830 ^t
	Within-group P-value	0.020^P		0.042^P					
ⁿ LH (IU/L)	Pre- treatment	30.69 \pm 27.09	19.73	29.57 \pm 22.07	22.05	1.127	-11.92	14.18	0.863 ^t
	Post-treatment	26.80 \pm 15.01	22.77	25.19 \pm 11.69	24.80	1.606	-5.509	8.72	0.653 ^t
	Change score	-3.89 \pm 20.51	-0.11	-4.37 \pm 22.85	-1.64	0.478	-10.93	11.89	0.933 ^t
	Within-group P-value	0.307 ^P		0.320 ^P					
ⁿⁿ E2 (Pg/mL)	Pre- treatment	25.13 \pm 12.35	20.00	27.06 \pm 12.92	20.55	-1.927	-8.577	4.72	0.197 ^{tt}
	Post-treatment	32.24 \pm 23.15	21.55	33.55 \pm 20.01	25.40	-1.316	-12.73	10.106	0.333 ^{tt}
	Change score	7.10 \pm 25.91	0.00	6.49 \pm 23.55	2.50	0.611	-12.444	13.667	0.772 ^{tt}
	Within-group P-value	0.108 ^w		0.066 ^w					
ⁿⁿ P (ng/mL)	Pre- treatment	0.698 \pm 0.75	0.49	0.61 \pm 1.13	0.26	0.087	-0.41	0.59	0.489 ^{tt}
	Post-treatment	0.377 \pm 0.31	0.28	0.310 \pm 0.18	0.24	0.067	-0.707	0.205	0.800 ^{tt}
	Change score	-0.321 \pm 0.81	-0.24	-0.300 \pm 1.18	0.03	-0.020	-0.552	0.511	0.273 ^{tt}
	Within-group P-value	0.049^w		0.524 ^w					
ⁿⁿ T (ng/mL)	Pre- treatment	0.271 \pm 0.14	0.20	0.341 \pm 0.405	0.20	-0.0707	-0.228	0.869	0.521 ^{tt}
	Post-treatment	0.297 \pm 0.26	0.20	0.240 \pm 0.095	0.20	0.056	-0.050	0.162	0.932 ^{tt}
	Change score	0.025 \pm 0.296	0.00	-0.101 \pm 0.376	0.00	0.127	-0.050	0.304	0.696 ^{tt}
	Within-group P-value	0.796 ^w		0.382 ^w					
ⁿⁿ E2/T	Pre- treatment	101.72 \pm 52.73	100.00	104.06 \pm 40.86	100.00	-2.332	-27.27	22.61	0.410 ^{tt}
	Post-treatment	128.63 \pm 80.18	100.00	155.83 \pm 106.96	117.00	-27.203	-76.71	22.303	0.194 ^{tt}
	Change score	26.90 \pm 104.15	10.00	51.77 \pm 99.81	12.72	-24.870	-78.605	28.864	0.488 ^{tt}
	Within-group P-value	0.067 ^w		0.010^w					
ⁿⁿ E2/P	Pre- treatment	88.55 \pm 90.55	63.33	114.32 \pm 91.91	87.12	-25.77	-73.78	22.24	0.202 ^{tt}
	Post-treatment	100.78 \pm 57.57	100.00	122.22 \pm 84.50	100.00	-21.43	-59.24	16.37	0.333 ^{tt}
	Change score	12.23 \pm 117.72	29.38	7.898 \pm 115.25	25.79	4.33	-57.01	65.67	0.858 ^{tt}
	Within-group P-value	0.309 ^w		0.350 ^w					
ⁿ FSH/E2	Pre- treatment	1.809 \pm 1.004	1.59	1.696 \pm 1.143	1.33	0.112	-0.452	0.678	0.691 ^t
	Post-treatment	2.189 \pm 1.521	1.87	1.955 \pm 1.244	1.65	0.233	-0.500	0.967	0.526 ^t
	Change score	0.379 \pm 1.367	0.43	0.259 \pm 1.830	0.25	0.120	-0.725	0.967	0.776 ^t
	Within-group P-value	0.139 ^P		0.461 ^P					
ⁿ FSH/P	Pre- treatment	148.50 \pm 140.75	83.81	172.37 \pm 191.39	114.95	-23.873	-111.83	64.085	0.589 ^t
	Post-treatment	190.14 \pm 130.81	166.34	191.14 \pm 98.94	188.15	-0.997	-62.34	60.34	0.974 ^t
	Change score	41.64 \pm 189.85	59.38	18.77 \pm 221.777	68.69	22.875	-85.49	131.24	0.674 ^t
	Within-group P-value	0.239 ^P		0.658 ^P					
ⁿ FSH/T	Pre- treatment	170.23 \pm 96.46	157.50	172.35 \pm 113.13	147.53	-2.111	-57.293	53.070	0.939 ^t
	Post-treatment	229.66 \pm 158.84	176.18	236.28 \pm 131.43	194.39	-6.624	-83.621	70.373	0.864 ^t
	Change score	59.42 \pm 153.57	43.17	63.93 \pm 182.73	42.47	-4.512	-93.088	84.06	0.919 ^t
	Within-group P-value	0.043^P		0.075 ^P					

Between-group p-values show the significance of differences in the studied parameters between study group before and after the trial period. Within-group p-values show the significance of differences in the studied parameters of each study groups before and after the trial period. Change score p-values compare between P-values of each studied parameter. n: Normal, nn: Non-normal, t: T-test; tt: Mann-Whitney test, w: Wilcoxon test, p:Pair t-test. Bold figures show that the difference is significant ($p < 0.05$). FSH, Follicle-stimulating hormone; LH, Luteinizing Hormone; E2, Estradiol; P, Progesterone; T, Testosterone.

models for understanding the physiology of human (Perlman, 2016; AKHTAR, A. 2015; Shanks et al., 2009; Varga et al., 2010; Worp et al., 2010).

Finally, *E. angustifolia* (Senjed) is a general fruit on Iranian table that is consumed by both male and female people from children to adult. In

Iranian folklore, Senjed is known as the symbol of love and fertility and also is a central part of Nowruz, Iranian traditional ceremony of new year (Bigonah et al., 2012; Intini, 2015; Michael, n.d.). Furthermore, it is postulated in Iranian traditional medicine that the fruit can increase sexual desire and improve menopausal complications (Akbarzadeh

et al., 2014; Matin, 2013).

5. Discussion

Due to the significant negative effects of menopausal symptoms (Al-Safi and Santoro, 2014), as well side effects and dangers associated with hormone therapy (Panay et al., 2013), most women tend to get the right treatment to eliminate these complications. In Iran, only 15% of menopausal females use hormone replacement therapy (Panay et al., 2013). There is a strong belief on the efficacy of *E. angustifolia* among Iranian groceries (unpublished gathered data from several groceries in Babol, Damghan, Shahroud, Tehran, Iran) and the general public in Iran (Matin, 2013; and personal observations of present authors). Protective and beneficial effects of *E. angustifolia* in various diseases have been studied, previously (Farzaei et al., 2015; Hamidpour et al., 2017; Khan et al., 2016; Nikniaz et al., 2014, 2016; Tehranizadeh et al., 2016). In this study, the effects of *E. angustifolia* on the sex hormones of menopausal women have been investigated for the first time.

The increase of FSH hormone in within-group analysis of herbal medicine ($p = 0.020$) and placebo ($p = 0.042$) groups was significant but changes between these groups was not significant (change score p -value = 0.830). The level of LH, the other pituitary hormone, decreased insignificantly in both within-group analyses of herbal medicine ($p = 0.307$), and placebo ($p = 0.320$) groups and in between-group analysis of study groups (change score p -value = 0.933). During menopause, an increase in the levels of LH and FSH, but a decrease in the production of estradiol is seen (Navarro-pardo et al., 2018; Yum and Kim, 2014). To reduce FSH, the levels of estradiol, inhibin B and progesterone should be increased. The hypothalamus increases the secretion of GnRH in response to the reduction of estradiol and progesterone and subsequently increases the amount of FSH (Honour, 2018). In this trial, the biological indices of GnRH and inhibin B were not measured.

Increasing estradiol leads to improvements in menopausal symptoms (Diem et al., 2018). Estradiols are potent activators of immune responses, especially humoral immunity. In the postmenopausal years, low levels of estradiol facilitates macrophage or T-cell associated diseases (Chiara et al., 2017). The changes in estradiol levels was not significant in both herbal medicine and placebo groups (within-group p -values = 0.108 and 0.066, respectively), as well between study groups (change score p -value = 0.772). However, around 28% increase was seen in estradiol level from 25.13 ± 12.35 at the beginning of the trial to 32.24 ± 23.15 after 10-weeks treatment with *E. angustifolia*. Accordingly, it can be expected that increasing estradiol after treatment with *E. angustifolia* can help to improve the level of immunity in postmenopausal women which further studies with a larger sample size in a longer time period are needed to examine it. Phytosterols, Isorhamnetin and its derivatives, kaempferol, quercetin, as well as β -Sitosterol and Stigmasterol, have been identified in *E. angustifolia*'s fruit and peel (Farzaei et al., 2015; Hosseinzadeh et al., 2003). To the best of our knowledge, absorption, bioavailability, and the metabolism of *E. angustifolia*'s isoflavons and other metabolites have not been studied, yet. However, it has been shown that gut microflora can convert isoflavones into active oestrogenic substances (Coxam, 2008; Gupta and Prakash, 2014). Accordingly, the observed changes in estradiol levels in our study can be attributed to the phytoestrogenic effect of *E. angustifolia*. Actually, the mode of action of phytoestrogens has some complexities such as their affinity to binding to progesterone and androgen receptors, and also the capacity of isoflavones to induce the biosynthesis of hepatic sex hormone binding globulin and changing the free fraction of endogenous circulating steroids. In fact, phytoestrogens are considered endocrine disruptors (Patisaul, 2017; Villaseca, 2012). In the present study, any adverse effects were not seen in the postmenopausal participants of herbal medicine group.

Progesterone has a repressive effect on the immune system and is said to increase the risk of infection with human papillomavirus (HPV), while estradiol reduces the sensitivity to HPV infection (Hellberg et al.,

2005). In within-group analysis of herbal medicine group, a significant decrease in progesterone level was seen ($p = 0.049$), however by analyzing the change score of progesterone, it was not significant between two herbal medicine and placebo groups (change score p -value = 0.273). It can be concluded that a 10-week consumption of *E. angustifolia* did not have either significant adverse or beneficial effects regarding to the levels of estradiol and progesterone.

Testosterone replacement therapy in menopausal women seem to improve sexual desire (Sanchez-Borrego et al., 2014). In our study, the amount of changes in testosterone was not significant (change score = 0.696). According to within-group analysis, the increase of testosterone was not significant in herbal medicine group ($p = 0.796$). However, around 9.6% increase of testosterone level from 0.271 ± 0.14 to 0.297 ± 0.26 after 10-week treatment with *E. angustifolia*. The satisfaction with sexual life was not evaluated in present trial. However, a significant increase of the sexual events, the orgasms and sexual desire, as well as a decrease in distress was seen after transdermal testosterone treatment (Sanchez-Borrego et al., 2014). Akbarzadeh et al. also showed the efficacy of *E. angustifolia* flower extract in the reduction of the frequency of orgasmic disorder in women (Akbarzadeh et al., 2014).

Statistical analysis of hormone ratios showed that the increase of the estradiol to testosterone ratio was not significant between two study groups after 10 weeks of trial (change score p -value = 0.488). However, the increase of both estradiol and testosterone was not significant after consumption of *E. angustifolia* (within-group p -values = 0.108 and 0.796 and percentages of changes = 28% and 9.6%, respectively), 26% increase of the hormone ratio from 101.72 ± 52.73 to 128.63 ± 80.18 (within-group p -value = 0.067 which is significant at a $p > 0.1$) might show that part of the reduction in testosterone in the herbal medicine group is also contributing to the increase in estradiol, this can be verified by measuring the activity of enzymes involved in converting testosterone to estradiol (Burdea and Mendez, 2019).

Increase in the proportion of estradiol to progesterone hormone is known a risk factor for cancer of the uterus (Honour, 2018) and is in line with managing the menopausal side effects (Monteleone et al., 2018) Despite of the significant decrease in progesterone (within p -value = 0.049) and insignificant decrease in estradiol (within p -value = 0.108), the increase in the ratio of estradiol to progesterone was not significant in both within-group analysis of herbal medicine group ($p = 0.309$) and between-group analysis (change score p -value = 0.858). Similarly, the increase in hormonal ratio of FSH to estradiol was not significant (change score p -value = 0.776) between two study groups in spite of the significant increase of FSH after consumption of *E. angustifolia* (within-group p -value = 0.020). The hormonal ratio of FSH to estradiol is an important criterion for assessing the effect of a drug on the side effects of menopause (Radwan et al., 2017). In present trial, changes in menopausal complications was not significant after the course having *E. angustifolia* and placebo, except for joint pain that significantly improved in herbal medicine group ($p = 0.0003$).

As previously shown, *E. angustifolia* shows anti-inflammatory and pain relieving effects (Farzaei et al., 2015; Hamidpour et al., 2017) and muscle relaxant activity (Hosseinzadeh et al., 2003). Postmenopausal participants of herbal medicine group also reported joint pain reduction ($p = 0.0003$). Part of the antinociceptive effects of *E. angustifolia*, a known source of flavonoids, is likely to be due to its anti-inflammatory properties. Some prostaglandins (PGs), eicosanoids derived from arachidonic acid, cause inflammation, fever and pain, and some tend to contract the smooth muscles of the uterus in menstruation. Some PGs also play a role in regulating the sleep cycle. Prostaglandins are biosynthesized by the action of cyclooxygenase (COX) isozymes (Ricciotti & Garret A, 2012). Aqueous extract of *E. angustifolia* could inhibit cyclooxygenases' activity and also reduce pain and inflammation induced by formalin in mice (Farahbakhsh et al., 2011). Flavonoids can

contribute in reducing pain and inflammation through the prevention of the production of prostaglandins from arachidonic acid by inhibiting the activity of calcium-dependent phospholipase A2 and the release of arachidonic acid from phosphatidic acid (Hosseinzadeh et al., 2003; Romano et al., 2013). On the other hand, by inhibiting inflammatory cytokines from activated macrophages in inflammation, the anti-inflammatory effect of flavonoids, such as inhibition of tumor necrosis factor production (TNF), is applied (Tokeret et al., 2004). Further studies are needed to clarify the effects of *E. angustifolia* on the function of immune system in menopausal women.

6. Conclusions

E. angustifolia improved joint pain and changed the sex hormone profile and the condition of menopausal participants to a limited extent which was not in a complete accordance with the folklore Iranian traditional medicine, possibly due to the small sample size and the short time of our study.

Funding

This clinical trial was supported by Damghan University (Damghan, Iran) and Alborz University of Medical Sciences (Karaj, Iran) according to Memorandum of Understanding of 4/4-190681.

Sponsor (s)

1- Damghan University, Damghan, Iran. 2- Alborz University of Medical Sciences, Karaj, Iran.

Clinical trials registry site

<https://www.irct.ir/>.

Clinical trials registration code

IRCT2017030932795N2.

Acknowledgements

We wish to express our thanks to Mrs. Maryam Mohammdbeygi and Dr. Alireza Mohammadyari (Iranzamin Laboratory, Karaj, Iran) for their invaluable assistance. We are sincerely thankful to Dr. Mehdi Yasari for his biostatistics guidance. We are grateful to all the menopausal mothers who accompanied us in this research. We wish to thank our family whom we spent much of our time belonging to them for our research and studies. We are also very thankful to Professor Shikov and the great reviewers for their invaluable role in the improvement of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2019.112229>.

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