Women. Health. Bull. 2023; 10(1)

Published online 2023 January.

**Randomized Clinical Trial**

# Effects of Oral Use of *Elaeagnus angustifolia* L. on the Genitourinary Laboratory Findings in Postmenopausal Women: A Randomized, Double-Blind Clinical Trial

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**Received:** September 05, 2022; **Revised:** October 22, 2022; **Accepted:** November 30, 2022

## Abstract

**Background:** Vaginal microbiota are believed to profoundly impact the overall quality of life. Moreover, reduced levels of circulating estrogen are responsible for the majority of the changes in the genital tract in postmenopausal women. Accordingly, the present study aimed to investigate the effects of *E. angustifolia* (EA) on the genitourinary system in postmenopausal women.
**Methods:** In this double-blinded, randomized, placebo-controlled trial, we randomly assigned 58 postmenopausal women to one of the two medicinal herb-receiving (15 g of whole EA fruit powder) and placebo-receiving (7.5 g of isomalt+7.5 g of cornstarch) groups. Initially and after 10 weeks of the treatment, urinalysis, vaginal microbial culture, and vaginal pH measurement were carried out. This study was registered in the Iranian Registry of Clinical Trials with the code of IRCT20170227032795N4. Additionally, qualitative and quantitative data were analyzed using the Chi-square and the ANCOVA tests, respectively.
**Results:** The findings revealed that a 10-week treatment with EA had no significant effects on urine pH (5.733±1.014; P=0.728), specific gravity (1.022±0.006; P=0.438), as well as the count of red blood cells (1.533±2.562; P=0.080), white blood cells (3.750±7.109; P=0.349), and epithelial cells (2.116±1.798; P=0.595), and the qualitative parameters, including protein (P=0.612), blood (P=0.261), nitrite (P=0.483), bacteria (P=0.179), and mucus (P=0.564).
**Conclusion:** Oral consumption of EA did not significantly change the studied parameters. Thus, further studies with larger sample sizes, longer duration, subjects of different age groups, and other routes of administration could be suggested.

***Keywords:*** *E. angustifolia*, Urogenital system, Urinalysis, Traditional Medicine, Iran

**How to Cite:** Rezaei A, Badehnoosh B, Mohammadbeigi M, Qorbani M, Emaminia F, Mohammadyari AR, Jalalvand F. Effects of Oral Use of *Elaeagnus angustifolia* L. on the Genitourinary Laboratory Findings in Postmenopausal Women: A Randomized, Double-Blind Clinical Trial. Women. Health. Bull. 2023;10(1):1-12. doi: 10.30476/whb.2023.97069.1203.

## 1. Introduction

The maintenance of human health depends on a symbiotic relationship between humans and the associated bacteria. Vaginal microbiota change dramatically during female life, which are associated with the hormonal and microanatomical characteristics of the vaginal epithelium (1). In addition, a set of microorganisms (bacteria and yeasts) colonize the vaginal site and creates a balanced vaginal ecosystem. That mentioned, reduced circulating estrogen levels are responsible for most of the anatomical, cytological, bacteriological, and physiological changes occurring in the female genital tract in postmenopausal women. Vaginal microbiota can profoundly impact vulvovaginal atrophy, vaginal dryness, sexual health, and the overall quality of life (1). Particularly in postmenopausal women, disturbances in the vaginal microbiota lead to their vulnerability to gynecological and sexually transmitted infections (2). The mechanisms of the protective effect of the microbiota on the vagina include alteration of vaginal pH (VpH), production of bacteriocins, and formation of surfactants and biofilms. Moreover, it has been noted that urinary tract infection (UTI) is virtually always preceded by vaginal colonization with urinary tract pathogens (3). The genitourinary syndrome of menopause (GSM) also includes genital symptoms

(dryness and irritation), sexual symptoms (pain or lack of lubrication), and urinary symptoms (urinary urgency, dysuria, and recurrent urinary tract infections). Most women who do not take supplemental vaginal estrogen find that GSM symptoms worsen with age (4).

Vaginal lactobacilli have a protective function, producing antimicrobial compounds, such as lactic acid and hydrogen peroxide, as well as a biosurfactant that inhibits the adhesion of uropathogens to surfaces and stimulates the nonspecific innate immune system. In some cases, menopause results in depletion of *Lactobacillus* spp. and an increase in various microbial species and communities associated with a greater extent of vaginal symptoms, such as vaginal atrophy, dryness, pH changes, and dyspareunia (1).

Certain treatments, such as non-hormonal vaginal therapy, vaginal and systemic hormone therapy, complementary and alternative medicine, and selective estrogen receptor modulator, have been proven effective in symptom relief and reversal of atrophic anatomic changes associated with menopause (5). Furthermore, there is some evidence on the efficacy of oral and vaginal probiotics and some herbal medicines, such as soy, black cohosh, and cranberries, on vaginal and reproductive hormones in menopausal women (1, 5).

*Elaeagnus angustifolia* L. (Russian olive), known as Senjed in Persian, is a well-known medicinal plant. Traditionally, it has been used as an analgesic, antipyretic, and diuretic herbal medicine. Of note, a large number of compounds have been extracted from Russian olive, making this plant a source of flavonoids, alkaloids, minerals, and vitamins (6). This medicinal plant is also the symbol of fertility, whose bloom is the symbol of love in Iranian folklore. Its consumption is highly recommended for women's health (7). That said, *E. angustifolia* (EA) extract has been reported to significantly improve female orgasmic disorder (8, 9). Additionally, the bactericidal activity of the crude methanolic extract and fractions from the leaves, flowers, fruits, and roots of EA against *Escherichia coli* and *Staphylococcus aureus* has been confirmed. A number of fractions (n-hexane and ethyl acetate) also showed antibacterial effects against *Pseudomonas aeruginosa* (10). On top of that, antifungal and antibacterial effects were reported in some cases, which varied depending on the dose used and the extraction method (11). In Iranian folk medicine, EA is attributed to various beneficial effects, and its use is recommended at all ages. In our previous studies, the effects of EA on sex hormones (7), glycemic and lipid profiles, and cardiovascular system (12), as well as thyroid-stimulating hormone (TSH), dehydroepiandrosterone-sulfate (DHEA-S), prolactin (PRL), and cortisol levels (13) were investigated in postmenopausal women. On account of the effects of nutrition on the body microbiome (14), the reported beneficial and antimicrobial effects of EA, and the importance of vaginal microbiota in the health of elderly women, this study investigated the effect of ripe EA whole fruit on the vaginal microbiome in postmenopausal women.

## 2. Methods

### *2.1. Study Design*

This double-blind, randomized, placebo-controlled study follows the CONSORT 2010 checklist of clinical trials (15). Initially, we got the approval of the ethics committee of the university, with the ethics code of Abzums.Rec.1396.162, and registered the study in the clinical trial system with the code of IRCT20170227032795N4. Subsequently, those referred to the gynecological clinic of Kamali Hospital, Karaj, Alborz province, Iran were invited to participate in the study according to the guidelines for inclusion and exclusion criteria, after confirmation of the gynecologist and a personal interview. We also explained the purpose and method of the study to the participants, who then signed an informed consent.

### *2.2. Major Inclusion and Exclusion Criteria for the Study*

The inclusion criteria were as follows: age ranging from 40-70 years and a serum total cholesterol level of 200-300 mg/ml with no need to medical treatment. On the other hand, individuals with cardiovascular or renal diseases, metabolic disorders, such as diabetes, as well as users of psychiatric drugs and those with destructive habits, like smoking cigarettes or hookah, and alcohol and drug use, were excluded from the study. We selected the women who had not taken antibiotics in the previous two months, or did not demonstrate daily use of or sensitivity to EA. The participants were subjected to an initial screening, checking their blood pressure, heart rate, total cholesterol,

body mass index (BMI), and follicle-stimulating hormone (FSH) to confirm menopause.

### *2.3. Randomization and Sample Allocation*

Out of the 150 eligible postmenopausal women presenting to the gynecology clinic at Kamali Hospital, 81 met all the eligibility criteria for the prospective trial, for whom Pap smear, vaginal culture, and pH were evaluated. Due to budget constraints, 60 patients were randomly selected from 81 who had met all eligibility criteria by drawing 60 pieces of paper (out of a bowl) with random numbers on; a completely uninvolved person chose these 60 papers. The first 30 pieces of paper indicated the placebo group while the second 30 were the herbal medicine group (16-18). Accordingly, the final sample size of 30 participants was chosen based on previous clinical trials (12-14, 16, 18). In our previous study, for TSH, considering the SD of 2.7 in each study group, the average difference of 2 between the two study groups, alpha and beta were 0.05 and 0.2, respectively, and the sample size was determined to be 29 (13). Figure 1 shows the flowchart of patient recruitment and retention.

### *2.4. Preparation of Treatments*

The fruits of EA were harvested in October 2017 from the gardens of Damghan city (Semnan province, Iran); they were dried completely in a suitable place and ground in three passes, turned into a smooth powder, as described previously (7). An herbarium specimen of EA (voucher number: Amirahmadi 1842 (DU000584)) was also kept in the herbarium of Damghan University. Cornstarch from Bijan Pharmacy (Tehran, Iran) and isomalt from Puyakabak Food Company (Tehran, Iran) were food grade. Moreover, the packaging of the herbal medicine and the placebo was conducted by a person who was not involved in this project; in that regard, they were put into two packages with different color schemes (red and blue, respectively). Of note, the individuals involved in the study, including the supervisors, the coauthors, project students, the employee of Iran-Zamin Lab, as well as the postmenopausal participants, were unaware of the nature of these packages until the end of designing, receiving the final test results, and performing the statistical analysis of the data.

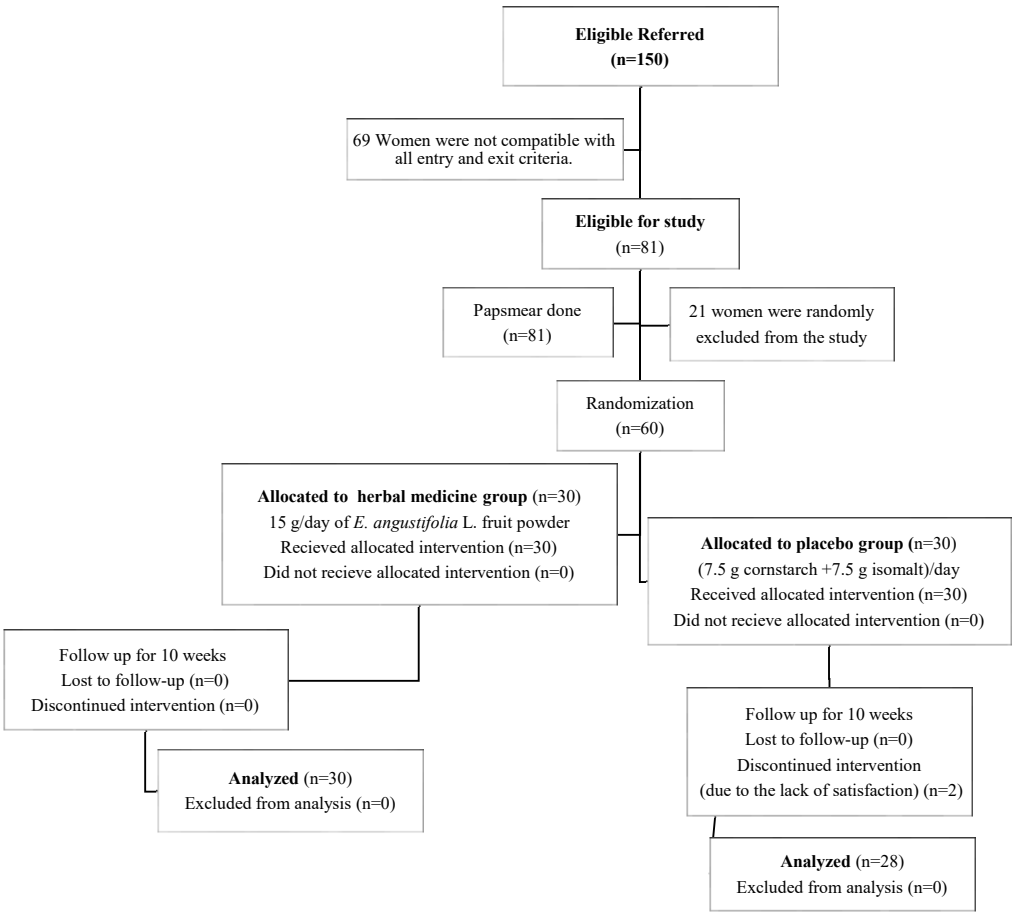


**Figure 1:** The figure shows the flowchart of the participants' enrollment, randomization, and retention.

*2.5. Treatment Dosage and Duration*

Two study groups of herbal medicine (n=30) and placebo (n=30) orally consumed 15 grams of ripe EA whole-fruit powder and placebo (15 grams of isomalt and cornstarch combined in a 1: 1 ratio) on a daily basis for 10 weeks. The participants were advised to consume the packs' contents after breakfast and with milk if possible (16). The packs were stored in a refrigerator before being given to the participants who were advised to store their packs in the refrigerator before consumption.

These dosages and the duration of consumption were chosen based on other clinical trials that examined the effects of EA, isomalt, and cornstarch on osteoarthritis (17), lipid profile and atherogenic profile in obese women (16), and the ineffectiveness of cornstarch and isomalt on blood glucose and lipid profiles (16, 19).

*2.6. Data Collection and Laboratory Analysis*

The main outcome measure was the urine pH (UpH) and culture while the secondary outcome measures included urine (appearance, blood, cell count, crystal, mucus, nitrite, and protein) and vaginal (pH, cell count) parameters. Data collection was performed by collecting fasting blood samples and completing an interview questionnaire. Data collection and laboratory tests were performed at Iran-Zamin Lab (Karaj) in two phases: before starting the herbal medicines/placebo and after 10 weeks of daily intake of the herbal medicines/placebo. Furthermore, the laboratory tests included: 1- urinalysis and microbial culture, 2- vaginal microbial culture and VpH measurement, and 3- Pap smear sample (liquid-based) collection.

A dipstick (urine test strip) (Med-Test, Germany) was used for urinalysis to determine pH, sugar, protein, urobilinogen, ketone bodies, and nitrite while a refractometer (Hand-Held Refractometer, ATAGO, Japan) determined the urine-specific gravity. In addition, the presence of protein was determined with a urine test strip (Urine test strips, Medi-Test, Germany). Protein positivity; on the other hand, was confirmed by adding 3% sulfosalicylic acid (19) (Merk, Germany). According to the direct report of a wet-mount smear of urine specimens, the urine deposit was also examined microscopically (19).

Microscopic examination of the urine sediment was studied based on the direct wet-mount smear of urine specimens (20). We performed the microbial culture utilizing sterile loops and under the hood in eosin methylene blue (EMB) (Merck, Germany) and blood agar media (Merck, Germany). That was carried out by incubating the plates at 37 °C for 24 hours. Furthermore, two types of sterile slides, including cotton swabs (two) and Dacron swabs (one), and a sterile Pap smear spatula were used to examine the cervix (21); one of the cotton swabs was used to prepare a direct spread on the slide, which was used for Gram staining.

Dacron swabs and other cotton swabs were utilized for microbial culture. The subcultures; on the other hand, were prepared from the culture on EMB on chocolate agar (Merck, Germany), blood agar (Merck, Germany), and EMB (Merck, Germany), and the thioglycolate tubes (Merck, Germany) were placed in an incubator at 37 °C for 24 hours. The samples cultured on chocolate agar and blood agar were incubated in an anaerobic vessel at 37 °C for 24 hours to test for anaerobic bacteria. For the plates with positive culture, the antibiogram disk diffusion procedure (the Kirby-Bauer disk diffusion susceptibility test) was performed using the agar diffusion disk method (Kerry Blair) while those with negative culture were reported as microbially negative (mast disks) (CLSI instructions (19). Additionally, for Pap smear, endocervical and ectocervical secretions were collected with a special spatula, and speculum were transferred to a liquid Pap smear. After centrifugation at 1200 g for 10 min, the sediment was homogenized in a 0.5-mL cellular base. Subsequently, 50 microliters of the sample was placed on a slide, which was examined using the Papanicolaou method after drying at room temperature (22).

*2.7. Statistical Analysis*

The effects of the treatments on the studied parameters were analyzed via SPSS version 25, with a significance level of $P<0.05$. Qualitative data, such as the urine color, appearance of the urine, and protein and blood in the urine were analyzed using the Chi-square test. For the expected counts of below and above 20%, the Chi-square test and Fisher's test were used, respectively. Meanwhile, quantitative data were analyzed utilizing the ANCOVA. The average was used when the

pretreatment and posttreatment data were not a unique number, but reported in a range; for example, 18-24. Since we only had the pretreatment data from the direct vaginal smear, the t-test was used, and the results were reported as the baseline data in the baseline table.

## 3. Results

Herein, we recruited postmenopausal women who were invited to the study according to the inclusion and exclusion criteria. The inclusion criteria were as follows: 1) menopause in the age range of 40-70 years; 2) the serum level of total cholesterol of between 200-300 mg/ml, with no need to medical treatment. The exclusion criteria however included: 1) metabolic disorders, such as diabetes; 2) cardiovascular and renal diseases; 3) having destructive habits, like smoking cigarettes and hookahs, or consuming alcohol and drugs; 4) those using psychiatric drugs.

Table 1 depicts the demographic information of our participants. Based on the results, no significant difference was seen between the postmenopausal participants regarding the demographic variables. The mean age of the postmenopausal participants was 56.63±5.43 and 54.07±6.90 years in the herbal medicine and placebo groups, respectively (P=0.121). Moreover, most of the participants in the herbal medicine (70%) and placebo (67.85%) groups were illiterate and under diploma, with the rest having a diploma or higher (P=0.86). Concerning their marital status, about 67% of the participants in the herbal medicine group and 75% of those in the placebo group were married; the rest were single (P=0.48). Furthermore, the majority of our participants, being 96.67% in the herbal medicine and 85.71% in the placebo groups, were housewife, compared to the rest who were employed (P=0.18).

Before the trial, 10 participants in the herbal medicine reported UTI symptoms (burning and itching) whereas only four participants had the symptoms after the 10-week treatment. Meanwhile, in the placebo group, the UTI symptoms were reported by six participants before and at the end of the trial. The UTI symptoms did not however change significantly in the two study groups (P=0.511).

Table 2 shows the pretreatment results of

**Table 1:** The participants' demographic characteristics in the herbal medicine and placebo groups

| Variables | Herbal Medicine (n=30) Number (%) | Placebo Group (n=28) Number (%) | P value |
|---|---|---|---|
| Mean age±standard deviation (years) | 56.63±5.43 | 54.07±6.90 | 0.121 |
| Educational level | | | 0.86 |
| Illiterate & under diploma | 21 (%70.00) | 19 (%67.85) | |
| Diploma and higher | 9 (%29.97) | 9 (%32.15) | |
| Marital status | | | 0.48 |
| Single | 10 (%33.33) | 7 (%25.00) | |
| Married | 20 (%66.67) | 21 (%75.00) | |
| Employment | | | 0.18 |
| Employed | 1 (%3.33) | 4 (%14.29) | |
| Housewife | 29 (%96.67) | 24 (%85.71) | |

Except for age, which was analyzed by t-test, the rest of the variables were compared by Chi-square test. The significance level was set at P<0.05.

**Table 2:** Comparison of some vaginal variables between the participants in the medicinal herb and placebo groups at baseline and before treatment initiation

| Variables | Herbal Medicine Group (n=30) | Placebo Group (n=28) | Mean Difference | 95% Confidence Interval of the Difference | | P value |
|---|---|---|---|---|---|---|
| | Mean±SD | Mean±SD | | Lower | Upper | |
| VpH | 7.75±1.08 | 7.55±0.59 | 0.196 | -0.270 | 0.663 | 0.403 |
| Epithelial Cells (Number) | 6.36±2.53 | 6.44±2.33 | -0.079 | -1.362 | 1.203 | 0.901 |
| Red Blood Cells (Number) | 6.26±5.88 | 6.96±8.23 | -0.697 | -4.443 | 3.047 | 0.710 |
| White Blood Cells (Number) | 6.00±7.26 | 4.28±6.27 | 1.714 | -1.869 | 5.297 | 0.342 |
| Yeast (Number) | 0.06±0.25 | 0.00±0.00 | 0.066 | -0.029 | 0.162 | 0.170 |

Smears prepared from Pap smear samples were stained by Papanicolaou method. The significance level was set at P<0.05.

VpH (P=0.403), the numbers of epithelial cells (P=0.901), red blood cells, RBC (P=0.710), white blood cells; WBC (P=0.342), and yeasts (P=0.170) in the vaginal samples of the postmenopausal participants in the herbal medicine and placebo groups. As seen, there was no significant difference between the two study groups in this regard.

Table 3 demonstrates the comparative results of the qualitative data in urinalysis and urine culture between the participants of the two study groups. After evaluating the differences between each of the studied parameters at the beginning and the end of the study, the efficacy of the herbal medicines was investigated. Accordingly, the urine color was yellow in both groups (herbal medicines and placebo) before and at the end of the study, with no change. The appearance of urine was assessed based on three characteristics, namely being clear, semi-clear, and semi-turbid. At the end of the 10-week study, the appearance of urine was significantly clearer in the placebo group than in the herbal medicine group (P=0.002). In addition, urine protein (P=0.612), nitrite (P=0.483), and blood (P=0.261) were reported as positive or negative. In terms of these parameters, there was no significant difference between the two study groups. Regarding the three conditions of "negative," "rare," and "positive" for the presence of bacteria in the urine sediment, no significant difference was found between the groups at the end of the study (P=0.179). Moreover, the presence of mucus, yeast, and crystals in the urine sediment was reported as negative, rare, and positive. There was no significant difference between the two groups regarding the presence of mucus in the urine (P=0.564). However, after 10 weeks of treatment, no yeast or crystals were found in the urine samples of the placebo group, which was significantly different with the result of the herbal

**Table 3:** Comparison of qualitative urinalysis and urine culture data between the participants in the medicinal herb and placebo groups at the beginning and after the 10-week daily treatment

| Variables | Category | Total | Groups | | | | Total | | P value* |
|---|---|---|---|---|---|---|---|---|---|
| | | | Herbal Medicine | | Placebo | | | | |
| | | | No. | Percentage (%) | No. | Percentage (%) | No. | Percentage (%) | |
| Appearance | Clear | 0 | 16 | 53.33 | 23 | 82.14 | 39 | 67.25 | 0.002[f] |
| | Semi-clear | 1 | 12 | 40 | 1 | 3.57 | 13 | 22.41 | |
| | Semi turbid | 2 | 2 | 6.67 | 4 | 14.28 | 6 | 10.34 | |
| Protein | Negative | 0 | 27 | 90 | 28 | 96.43 | 55 | 93.10 | 0.612[f] |
| | Positive | 1 | 3 | 10 | 1 | 3.57 | 4 | 6.90 | |
| Blood | Negative | 0 | 23 | 76.67 | 23 | 82.15 | 46 | 79.31 | 0.261[f] |
| | Rare | 1 | 6 | 20 | 2 | 7.14 | 8 | 13.79 | |
| | Positive | 2 | 1 | 3.33 | 3 | 10.71 | 4 | 6.90 | |
| Nitrite | Negative | 0 | 30 | 100 | 27 | 96.43 | 57 | 98.28 | 0.483[f] |
| | Positive | 2 | 0 | 0.0 | 1 | 3.57 | 1 | 1.72 | |
| Bacteria | Negative | 0 | 17 | 56.67 | 19 | 67.86 | 36 | 62.07 | 0.179[c] |
| | Rare | 1 | 9 | 30 | 3 | 10.71 | 12 | 20.69 | |
| | Positive | 2 | 4 | 13.33 | 6 | 21.43 | 10 | 17.24 | |
| Mucus | Negative | 0 | 24 | 80 | 22 | 78.58 | 46 | 79.31 | 0.564[f] |
| | Rare | 1 | 5 | 16.67 | 3 | 10.71 | 8 | 13.79 | |
| | Were seen | 2 | 1 | 3.33 | 3 | 10.71 | 4 | 6.90 | |
| Yeast | Negative | 0 | 23 | 76.67 | 28 | 100 | 51 | 87.93 | 0.016[f] |
| | Rare | 1 | 1 | 3.33 | 0 | 0 | 1 | 1.72 | |
| | Were seen | 2 | 6 | 20.0 | 0 | 0 | 6 | 10.35 | |
| Crystal | Negative | 0 | 23 | 76.67 | 28 | 100 | 51 | 87.93 | 0.016[f] |
| | Rare | 1 | 1 | 3.33 | 0 | 0.0 | 1 | 1.72 | |
| | Were seen | 2 | 6 | 20.0 | 0 | 0.0 | 6 | 10.35 | |
| Urine culture | Negative | 0 | 28 | 93.33 | 28 | 100 | 56 | 96.55 | 0.492[f] |
| | Positive | 1 | 2 | 6.67 | 0 | 0.0 | 2 | 3.45 | |

c: Chi-square test; f: Fisher test. Bold figures show that the difference is significant (P value < 0.05). Only the change scores of the variables have been reported. For instance, to calculate the changes in crystals, their type was unimportant. Changes in amount were considered. If any kind of crystals (many or few) were observed in a urine sample before the treatment and the crystals reduced or disappeared completely after the treatment, this positive change was indicated by number one. But if many urine crystals remained at the end of the trial, this no-change event was indicated by zero. When there were no crystals in urine samples before the treatment, but after taking the treatments, few or many crystals were seen, this negative change was indicated by number two.

**Table 4:** Comparison of quantitative urinalysis and urine culture data between the participants in the medicinal herb and placebo groups at the beginning and after the 10-week daily treatment

| Variables (unite) | Time | Herbal Medicine Groups (n=30) | Placebo Groups (n=28) | P value |
|---|---|---|---|---|
| | | Mean±SD | Mean±SD | |
| Specific Gravity | Pre-treatment | 1.021±0.009 | 1.024±0.006 | 0.63[1]a |
| | Post-treatment | 1.022±0.006 | 1.024±0.006 | |
| pH | Pre-treatment | 5.616±0.897 | 5.732±0.865 | 0.887 [a] |
| | Post-treatment | 5.733±1.014 | 5.750±1.075 | |
| Red Blood Cells (Number) | Pre-treatment | 3.550±5.686 | 1.839±2.844 | 0.488 [a] |
| | Post-treatment | 1.533±2.562 | 1.785±2.473 | |
| White Blood Cells (Number) | Pre-treatment | 4.583±6.887 | 3.071±6.538 | 0.305 [a] |
| | Post-treatment | 3.750±7.109 | 3.571±7.194 | |
| Epithelial Cells (Number) | Pre-treatment | 3.383±6.110 | 3.232±3.365 | 0.275 [a] |
| | Post-treatment | 2.116±1.798 | 2.675±2.138 | |

a: ANCOVA. The significance level was set at P<0.05.

medicine group in this regard (both P=0.016). Additionally, as for the microbial culture of the urine, there was no significant difference between the two groups at the end of the study (P=0.492).

The changes in quantitative data in urinalysis and culture between the two study groups before and after the treatment are represented in Table 4. Obviously, the level of the studied parameters, including specific gravity (P=0.631), pH (P=0.887), RBC (P=0.488), WBC (P=0.305), and epithelial cells (P=0.275), remained unchanged after the 10-week treatment with EA.

## 4. Discussion

The present double-blinded, randomized, placebo-controlled study is the first clinical trial on the efficacy of EA consumption on the genitourinary system in postmenopausal women. Herein, all the participants were similar regarding the demographic variables. At the beginning of the study and before the treatments, two study groups were similar and not significantly different in terms of VpH and numbers of epithelial cells, RBC, WBC, yeast, and fungi in the vaginal samples. Based on the obtained results, oral consumption of EA had no significant effects on the parameters studied, including urine pH, specific gravity, and count of RBC, WBC, and epithelial cells, as well as qualitative parameters, including protein, blood, nitrite, bacteria, and mucus.

Genitourinary microbiome (GM) may fluctuate with age and has pivotal roles in the discriminants of health and disease (14). In addition, both urinary and vaginal microbiota change over time in female (23). Compared to gut microbiota or the effect of hormone replacement therapy (HRT) on the vaginal microbiomes, little is known about the effects of nutrition or oral consumption of herbal medicine on GM, especially in postmenopausal women. In the present work, the baseline UpH levels of the herbal medicine and placebo groups were similar, with both study groups' lowest and highest UpH before and after the treatment being 5.616±0.897 and 5.750±1.075, respectively. Moreover, EA intake had no significant impact on UpH which reflects acid– base balance and relates to dietary acid –base load, fruit, vegetables, and meat intakes. Of note, the median pH of human 24-h urines is ~6 (24). Lower UpH is associated with an increase fasting blood sugar (FBS), glycated hemoglobin (HbA1c), serum triglyceride (TG), and a decrease in high-density lipoprotein cholesterol (HDL-C) (25). In this regard, Shabani and colleagues showed that oral EA significantly decreased serum level of low-density lipoprotein cholesterol (LDL-C) and HDL-C. However, the decrease in TG and the significant increase in FBS and HbA1C following the consumption of EA were not clinically significant (12).

Generally, the VpH at fertile age is 4-5, and the microbiota mainly consist of *Lactobacilli*. Nonetheless, at menopausal age, the microbiota change and consist of various mixed microbes, resulting in a VpH of 6 or 6.5 (5). In the current study, only the pretreatment VpH of the participants was evaluated, which was 7.75±1.08 and 7.55±0.59 in the herbal medicine and placebo groups, respectively.

No significant difference was observed in

urinalysis and culture between the two study groups. The only differences concerned the appearance of the urine, which was significantly clearer in the placebo group compared with that in the herbal medicine group at the end of the study, as well as the absence of yeast or crystals in the urine samples of the placebo group, which was significantly different from the herbal medicine group. It is noteworthy that the presence of pus, RBCs, or bacteria may result in a cloudy urine. Of course, normal urine may also be cloudy because of consumption of certain foods (26). Since the two study groups were similar in terms of bacteria in their urine analysis, the observed significant difference may be due to the ingestion of EA which is rich in minerals, vitamins, and various secondary metabolites (6, 27, 28). The few crystals observed in two of the participants' urine sample in the placebo group were calcium oxalate before and after the trial. However, in the herbal medicine group, a few amorphous phosphate crystals were seen in only one urine sample at the beginning of the trial. Nevertheless, at the end of the trial, a few amorphous and calcium oxalate crystals were observed in three and two samples, respectively. The type of crystals varies with the UpH and disease, with calcium and phosphate oxalate crystals occurring in the urine of patients with malabsorption states or parathyroid abnormalities (26). In this research, the participants were not evaluated for these abnormalities. EA fruits are rich sources of minerals, such as calcium, magnesium, potassium, iron, and manganese, with its calcium content being 36.18-42.27 mg/ 100g (28). Diets influence the urine composition and the risk of calcium oxalate crystallization (29, 30). However, EA is a common fruit in Iranian diet without any side effects (27), and since the changes in UpH was not significant, the observed crystals might be due to EA ingredients and not necessarily a disease. Further investigation is however necessary.

In the present trial, *Cocci* bacteria were seen in all the participants' vaginal samples. In comparison, *Lactobacilli* was seen in only five people from each study group, *Staphylococcus* was also seen in five and two participants in the herbal medicine and placebo groups, respectively (data not shown). The microbiomes of the bladder and urethra however differ from one to another. Female urine samples are dominated by *Prevotella*, *Lactobacillus*, and *Anaerococcus* (15, 31). *Lactobacillus* is a dominant member (over 97%) of the female lower reproductive tract (32), which seems to be important members of the healthy urine microbiome (30). The proportion of *Lactobacillus* in women over 55 years of age decreases by three times possibly due to the hormonal changes after menopause (14). A low relative abundance of *Lactobacillus* is associated with vulvovaginal atrophy, vaginal dryness, and decreased libido (32) in menopausal and postmenopausal women. In the current study, sexual satisfaction of the participants was not evaluated and the postmenopausal women reported no significant changes in vaginal dryness, possibly due to the oral consumption of EA, the short duration of the study, and their age.

Several factors, including stress, inflammation, age, exercise, antibiotic use, and diet type have been associated with the changes in microbiota (33, 34). Furthermore, estrogen levels play an important role in maintaining vaginal health by maintaining tissue integrity and normal function of vaginal microbiota (5). That said, estrogen therapy reverses vaginal atrophy by lowering VpH and vaginal dryness, thickening and revascularizing the vaginal epithelium, increasing vaginal secretions and the number of superficial cells, decreasing recurrent UTIs, and restoring normal vaginal microbiota (5). Moreover, transdermal testosterone treatment significantly increases the sexual desire and the orgasms (12, 35). In this regard, Emaminia and colleagues reported that estradiol and testosterone levels increased by approximately 28% and 9.6%, respectively, after a 10-week treatment with EA; these changes were not however significant. The participants did not notice any changes after receiving EA treatment and placebo in terms of complications, including hot flashes, sleep disorders, fatigue, urinary urgent, anxiety, and mood change. However, EA consumption significantly improved joint pain (7).

The effectiveness of several herbal medicine on genitourinary system has been investigated. The 12-week application of chamomile vaginal gel (36) and eight-week application of licorice vaginal cream (35) significantly improved vaginal burning, dyspareunia, dryness, and itching in postmenopausal women whereas orally administered fennel for 90 days was not effective in improving vaginal atrophy (37). Ghorbani and co-workers study on postmenopausal women with genitourinary syndrome showed that the oral consumption of ginseng had no significant effect on

vaginal maturation index and pH after four weeks (38). The sample size of the mentioned studies, ranging from 28 to 32 participants, was close to ours. The controversial outcomes of different studies may be due to the nature of the treatments, the route of administrations, and the duration of the studies. Based on the literature search, there are no studies on the efficacy of herbal medicines on microbiota in postmenopausal women. Moreover, the pharmacokinetic and pharmacodynamic of EA have not been fully studied. The current paper is among the first studies evaluating the efficacy of an herb on genitourinary laboratory findings, including vaginal and urine microbiota in postmenopausal women.

In the present study, the parameters studied did not change significantly after 10 weeks of treatment with EA. It has been pointed out that a longer phytoestrogen exposure is necessary to show positive effects on the vaginal epithelium and the resulting dryness (39). Based on the comparison of studies on the efficacy of estrogen therapy (5) and 28% increase in estrogen levels after treatment with EA (7), it can be concluded that the non-significant changes observed may be on account of the route of oral administration the treatment. Compared to the oral administration of medication, a vaginal route is more effective for local and systemic therapy (7). Moreover, most herbal studies on genitourinary system function have been conducted over a longer period than the present 10-week study. Furthermore, the participants in the current study were of postmenopausal age. Further investigation over longer time periods and with larger sample sizes, population in different age groups, and routes other than oral intake are required to evaluate the effectiveness of EA on genitourinary system.

Some parameters, including urine appearance and the presence of yeast or crystals in the urine samples changed significantly in the placebo group at the end of the study. Previous studies conducted in women with knee osteoarthritis (17) and obese women (16) reported that cornstarch and isomalt did not affect glycemic and lipid profiles. In our previous studies (13, 14), the placebo had significant effects on some parameters, such as total protein and triglycerides, which might be due to the differences in the sex and age of the study participants. In addition, no detailed information on the grade and sources of isomalt and cornstarch was provided in the aforementioned articles. Accordingly, further research is needed to clarify the suitability of isomalt and cornstarch as the placebo in postmenopausal women.

### *4.1. Limitations*

The sample size and the duration of the study are the main limitations of the present trial. Some herbal effects need more time to manifest. Additionally, post-treatment Pap smear results were not available for this 10-week trial. However, considering the promising effects observed for EA in the current and previous studies, the evaluation of its effects and mechanisms is worth further investigation.

## 5. Conclusions

The present study, for the first time, evaluated the effect of oral EA on the genitourinary laboratory findings in postmenopausal women. Unlike the placebo (isomalt+cornstarch), the 10-week intake of EA did not have any significant effects on the parameters assessed herein. A long-term study with a larger participant group or administration of EA in routes other than oral is needed to evaluate the effect of EA on urogynecological system function. The suitability of isomalt and cornstarch as a placebo in postmenopausal women also needs further investigation.

**Conflict of Interest:** None declared.

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