Original Article

The Effect of Citrus Aurantium Vaginal Cream on Vaginal Atrophy in Postmenopausal Women: A Quasi-experimental Study

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Abstract

Background: Vulvovaginal atrophy (VVA) is a common condition and a silent epidemic affecting many postmenopausal women who suffer from it in silence. This study aimed to evaluate the effect of Citrus aurantium vaginal cream on vaginal atrophy in postmenopausal women.

Methods: This single-group pretest-posttest quasi-experimental study was conducted on 30 postmenopausal women who were referred to the Gynecology Clinic of Imam Khomeini Hospital in the city of Noor, Iran, from June to November 2020. Citrus aurantium vaginal cream was administered to women diagnosed with vaginal atrophy (based on subjective symptoms of atrophy, descriptive evaluation of the vagina, vaginal pH measurement, and degree of vaginal maturation determined by vaginal smear) every night in the first two weeks and every other night for the second two weeks. Data were collected using the scale of subjective symptoms of vaginal atrophy; descriptive evaluation checklist of vaginal mucosa; laboratory results registration form (vaginal maturation index, vaginal maturation value, and vaginal pH) before the intervention and two and four weeks after the intervention. Data were analyzed using SPSS software (version 24) through the analysis of variance with repeated measurements, and LSD post-hoc test. A P value less than 0.05 ($P<0.05$) was considered statistically significant.

Results: Citrus aurantium vaginal cream improved subjective symptoms of vaginal atrophy ($P<0.001$), reduced the score of descriptive evaluation of vaginal mucosa ($P<0.001$), decreased vaginal pH ($P<0.001$), and increased vaginal maturity ($P<0.001$).

Conclusions: The results showed that citrus aurantium vaginal cream could improve the symptoms of vaginal atrophy without causing serious complications. However, further studies with a control group are suggested to confirm the findings of this study.

Trial Registration Number: IRCT20200215046494N1

Keywords: Atrophy, Citrus aurantium, Menopause, Vagina, Vaginal cream

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Introduction

Vulvovaginal atrophy (VVA) is a distressing medical condition and a silent epidemic that occurs due to ovarian depletion and aging.[1, 2] This common condition affects about 50-60% of postmenopausal women who suffer from this disease in silence, and over time, its frequency increases up to 73-75%.[2, 3] VVA is a major component and an integral part of Menopausal Genital Syndrome (GSM) due to decreased serum levels of estrogen and other sex steroids.[1,2,4] The most common signs and symptoms of VVA include vaginal dryness, dyspareunia, redness, itching, and occasional discharge and/or bleeding.[5] VVA is often underreported since talking about vaginal health is still regarded as a taboo subject. Many healthcare providers do not talk to their patients about this, and many women are not aware of the treatment.[4]

The lack of discussion on issues related to vaginal health ultimately leads to poor access to effective treatments.[4] Only 54% of postmenopausal women were willing to talk to a specialist about their sexual health, and 33% of patients even avoided answering questions in this regard.[2]

Due to the recent increase in life expectancy, women spend the last one-third of their lives (approximately 20-30 years) in menopause.[6, 7] Since vaginal atrophy has profound negative impacts on women's sexual health, vaginal dryness, dyspareunia, and discharge, it is important to treat postmenopausal women with vaginal atrophy.[8] The selection of treatment for vaginal atrophy depends on several factors, such as disease severity, patient preference, and effectiveness and safety of treatment.[8]

The chronic nature of VVA/GSM suggests that effective treatments should preferably be prescribed at the onset of signs and symptoms of vaginal atrophic changes and should be continued over time to maintain their benefits.[5] The application of non-hormonal vaginal lubricants and moisturizers as well as regular sexual activity should be the first line of treatment in reducing the VVA symptoms.[9] Hormone replacement therapy (HRT) can be considered the second line of treatment. Systemic HRT is prescribed when symptoms of genitourinary tract atrophy appear in combination with other symptoms of climacteric syndrome. Based on evidence, systemic HRT eliminates the symptoms of vaginal atrophy in 75% of cases, while topical treatment eliminates these symptoms in 80% to 90% of cases.[2]

Many women do not take estrogen therapy because of the side effects, including high risks of heart disease, stroke, pulmonary embolism, and breast cancer. Phytoestrogens can be used as an alternative therapy for these women. Phytoestrogens are plant compounds with estrogenic properties whose safety has been approved for many years. Soybean, fenugreek, red clover, and licorice plants are important sources of phytoestrogen.[10]

Citrus aurantium (Bitter orange) belongs to the Rutaceae family and is one of the most widely used native plants in Iran. In Iran, it grows in different regions of the country, such as Gilan, Mazandaran, Fars cities.[11] Citrus aurantium contains such compounds as limonene, linalool, β-myrsen, p-synephrine, and flavonoids.[12] Flavonoids contain a type of phytoestrogen found in plants such as bitter orange, fenugreek, licorice, soybean, and red clover and have estrogenic and antiestrogenic properties, which in menopause due to the low level of estrogen causes an increase in the level of estrogen in the blood circulation.[13, 14] The results of a study showed that inhalation of the scent of citrus aurantium improved the menopausal symptoms, increased libido, decreased cortisol, and increased blood estrogen.[15] No harmful or dangerous side effects have been reported on the appropriate application of the therapeutic dose of citrus aurantium.[16]

Since vaginal atrophy among postmenopausal women may be related to estrogen deficiency, citrus aurantium, as a natural product with estrogenic properties, may be administrated as a well-tolerated and efficacious treatment for relieving

vaginal atrophy. Given the importance of treating vaginal atrophy in postmenopausal women and studies conducted on the effect of phytoestrogen compounds on the improvement of symptoms, and regarding the lack of studies on the effect of citrus aurantium on the treatment of vaginal atrophy, this study aimed to determine the effect of citrus aurantium vaginal cream on the symptoms of vaginal atrophy in postmenopausal women.

Methods

The present study had a single-group pretest-posttest quasi-experimental design. The reason for not having a control group was the coincidence of sampling with the outbreak of the Covid-19 disease, which made recruitment phase difficult. This study was performed on postmenopausal women who were referred to the Gynecology Clinic of Imam Khomeini Hospital in the city of Noor, Iran, from June to November 2020.

The sample size was calculated 21 people, using G*Power version 3.1.9.2 software and based on the mean and standard deviation of the same study[10] (Mean of difference=6.75; SD of difference=7.80) and considering the confidence interval of 99% and the power of the test 90%. Given an attrition rate of 50% (due to the corona epidemic and the prediction of a severe drop of samples), the final sample size of 32 people was determined.

In this study, 120 women were evaluated for eligibility; of them, 88 women were excluded from the study due to: non-compliance with the inclusion criteria (n=78) and refusal to participate (n=10), so 32 women were included in the study. During the study, two women were excluded due to irregular use of the cream (n=1) and failure to visit at the appointed time (n=1). A total of 30 women completed the study.

Inclusion criteria were amenorrhea for at least one year unrelated to pregnancy, lactation, and other hormonal disorders, an age range of 45-65 years, the presence of sexual activity in the last 6 months, monogamy, lack of using too many phytoestrogens (e.g., soybean, red clover, and fenugreek) for eight weeks before the study, lack of vaginal infection (Candida, Trichomonas, and bacterial vaginosis) according to vaginal exam or sexually transmitted diseases based on the history obtained from the patient, lack of using HRT for eight weeks before the study, normal pap smear for the last three years (self-reported), lack of using vaginal medications or lubricants for at least 15 days before the study, lack of breast mass, uterine bleeding or spotting, lack of grade 3 or more pelvic organs prolapse, no history of asthma and allergy to certain plants or citrus, body mass index less than 30 kg/m^2, complaint of vaginal atrophy symptoms (e.g., vaginal burning, vaginal itching, vaginal dryness, and pain during intercourse), the existence of at least one vaginal atrophy symptom with a score of 65 or higher on the Visual Analogue Scale (VAS), the presence of at least two symptoms from the table of descriptive evaluation of vaginal mucosa (regardless of its severity), vaginal pH>5, and vaginal maturation value (VMV)<50.

However, those who consumed citrus aurantium vaginal cream less than 70% of the total number of times (namely, less than 14 times out of the total 21 times of cream use), had signs of allergy to the cream during the study, used phytoestrogens and vaginal drugs or any lubricant during the study, had uterine bleeding or spotting of unknown cause, had a vaginal infection during the study, and failed to visit the clinic on the designated days were excluded from the study.

Data collection tools included demographic characteristics form (age, mother's education and occupation, spouse's education and occupation, income level, body mass index, and history of drug sensitivity); obstetrics and gynecology information form including a) information about menstruation and menopause (i.e., the age of the first menstruation, age of menopause, duration of menopause, type of treatments used for genitourinary complications), b) information

about pregnancy (i.e., the number of pregnancies, the number of deliveries, age of the youngest child), c) information about intercourse (i.e., the number of intercourses per month); scale of subjective symptoms of vaginal atrophy; descriptive evaluation checklist of vaginal mucosa for clinical diagnosis of vaginal atrophy; laboratory results registration form (vaginal maturation index, vaginal maturation value and vaginal pH); daily drug use registration form; drug side effect registration form; and registration form of satisfaction with vaginal cream use.

Obstetrics and gynecology information form was created by the researchers, and their validity was determined based on content validity. In this way, at first, these forms were prepared using reliable sources and new articles, and then they were given to 10 faculty members (reproductive health specialists and gynecologists and obstetricians); then, the amendments were made according to the opinion of these professors.

The self-reported scale for subjective symptoms of vaginal atrophy assesses four criteria, including vaginal burning, vaginal itching, vaginal dryness, and dysparunia.[17] The severity of each symptom was measured using the VAS, consisting of a 100 mm line on which 0 mm indicated no symptoms and 100 mm indicated the most severe symptoms. The patient was asked to mark a sign on the line based on her perception of the severity of the symptoms.[3] The distance from the zero point to that sign was measured, and the atrophic signs with the highest numbers were considered to be the most bothersome symptom (MBS) for the patient. The total score was calculated by adding up the numbers obtained for each symptom.[3]

The validity of this tool has been confirmed in previous studies on menopause that have been conducted inside and outside of Iran.[18-20] The validity of the tool used in this study was confirmed using content validity and the opinion of 10 experts (reproductive health specialists, gynecologists and obstetricians). The reliability of the scale of subjective symptoms of vaginal atrophy was determined using the parallel reliability method. The reliability of this form was confirmed through the calculation of the correlation coefficient between the results (r=0.87).

The descriptive evaluation checklist of vaginal mucosa includes 5 items: vaginal color, rugae, petechia, elasticity, vaginal dryness. It was done by the first researcher in the presence of a gynecologist. Based on the severity of the signs, each sign was graded on a four-point scale from 0 to 3 (0=asymptomatic, 1=mild, 2=moderate, 3=severe). The total score of this checklist was calculated by adding up the individual scores obtained for the five items.[17]

The validity of this tool has been confirmed in previous studies on menopause that have been conducted inside and outside of Iran.[17-20] The validity of this tool in this study was confirmed using content validity and the opinion of 10 experts (reproductive health specialists, gynecologists, and obstetricians). The reliability of the descriptive evaluation checklist of vaginal mucosa was determined using the parallel reliability method. The reliability of this form was confirmed through the calculation of the correlation coefficient between the results (r=0.91). The laboratory results registration form (vaginal smear results and vaginal pH) had three parts: information about the percentage of the superficial, intermediate and parabasal cells (VMI), VMV, and vaginal pH.[3]

The validity of this form has been confirmed in previous studies on menopause that have been conducted inside and outside of Iran.[18-20] The validity of the tool used in this study was confirmed using content validity and the opinion of 10 experts (reproductive health specialists, gynecologists, and obstetricians).

Two samples from five research units were prepared before treatment and sent to the laboratory with different names at the same time to determine the reliability of laboratory colleagues. The correlation coefficient between the results was then determined (r=0.81).

The citrus aurantium vaginal cream was produced using dried citrus aurantium plant that has been prepared from commercial sources in the market, and its identity was confirmed at the herbarium of Mashhad University of Medical Sciences, Mashhad, Iran (herbarium number:13567). To prepare the hydroalcoholic extract of citrus aurantium, the dried citrus aurantium plant was pulverized by an electric grinder. Subsequently, 300cc of ethanol alcohol 70% was added per 100 grams of powder, and the mixture was poured into a special container with a glass lid and placed in an oven at 37 °C for 72 h. This mixture was stirred four to six times a day and then filtered using a Buchner funnel and Whitman filter paper. The solvent was removed from the extract using a rotary evaporator, at very low pressure and 37 °C, and the concentrated extract was obtained in the pharmacology laboratory of the medical school. Eventually, citrus aurantium cream (4%) was prepared by mixing the concentrated extract of citrus aurantium with Farabi base cream.

The prepared cream was poured into 50 g aluminum tubes using a special funnel, and the tubes were sealed and labeled after cooling. All cream preparation steps were performed under the laminar flow hood to maintain aseptic conditions throughout the process. Farabi base cream, which is available in the Iranian pharmaceutical market and is widely used for cream preparation, was produced by Farabi Pharmaceutical Company in Iran. The Farabi cream is a standard base cream with a production license that can be easily purchased and used by any researcher. The ingredients of Farabi base cream include Cetostearyl alcohol, petroleum gel, glycerin, mineral oil, preservative, and antioxidants.

The data collection was done through the completion of a checklist for postmenopausal women who were referred to the Gynecology Clinic of Imam Khomeini Hospital in the city of Noor, Iran. In order to confirm the diagnosis of vaginal atrophy in research units (including inclusion criteria), the self-reported scale for subjective symptoms of vaginal atrophy (vaginal burning, vaginal itching, vaginal dryness, and dysparunia) was completed first. In symptomatic women, the severity of the symptoms was measured by a VAS (a 100 mm line on which 0 mm indicates no symptoms and 100 mm indicates the most severe symptoms). In cases of acquisition a score of ≥65 for at least one of these symptoms, a gynecological examination was performed. If the vagina and cervix were normal (in terms of infection, cervicitis, lesion, and abnormal secretions), the descriptive evaluation checklist of vaginal mucosa by the first researcher and in the presence of a gynecologist, was completed. If at least 2 out of the 5 signs in the checklist got a minimum score of 1, the vaginal pH was checked by holding 2cm of pH strip (by a pair of pliers) against the lower third of the lateral vagina walls for 1 min. The color change of the pH strip was then compared with the standard scale on the strip box. In the cases with vaginal pH>5, a smear test was performed to determine the VMI. In this way, a sample of vaginal cells was obtained by scraping a plastic spatula against the lateral wall of the vagina on the surface of the cervix and placing the spatula on a slide. The sample was then immediately fixed and sent to a single pathology laboratory on the same day. After the sample was stained by Papanicola method by the pathologist, the percentage of superficial, intermediate, and parabasal cells (VMI) was determined. Then VMV was calculated based on: VMV=(percentage of superficial cells×1)+(percentage of intermediate cells×0.5)+(percentage of parabasal cells×0).[3] VMV less than 50 was the criterion for entering the study. If all inclusion criteria and diagnosis of atrophy were met, the patient was called to the clinic through a phone call to receive interventions. All sampling steps were done by the first researcher in the presence of a gynecologist.

In the first visit, a demographic questionnaire was completed for the participants. Then, they were given two tubes of citrus aurantium vaginal cream (50 g) and were asked to apply one applicator inside the

vagina every night before going to bed for two weeks. The participants were instructed to wash the applicator with lukewarm water and soap after using the interventions to prepare it for the next usage. In addition, they were also asked not to take any hormonal drugs or other vaginal compounds during the study. They were also advised to store the cream at a temperature of 25 degrees and in a dry environment away from sunlight. A daily drug use registration form was provided to the participants to be completed during the treatment period. The dates of the second and third visits were determined to be the 14th and 28th days of intervention, respectively. On the 14th day visit, a tube of vaginal cream (50 g) was given to the patient and she was taught how to take the medicine every other night for two weeks. On the 14th and 28th days, a re-examination was performed according to the self-reported scale of subjective symptoms of vaginal atrophy, descriptive evaluation checklist of the vaginal mucosa, vaginal pH, VMI, and VMV.

The participants were instructed to stop the treatment process in case of any possible side effects during the treatment period and call the researcher or refer to the clinic. During the study, the participants were contacted twice a week to be informed about the cream application and follow-up times, and they were asked not to use any hormonal drugs or vaginal compounds during the study period.

Data were analyzed through SPSS software (version 24), using the analysis of variance with repeated measurements and LSD post-hoc test. A P-value less than 0.05 was considered statistically significant.

The study protocol was approved by the Research Ethics Committee of Gonabad University of Medical Sciences, Gonabad, Iran (IR.GMU.REC.1398.142). The informed written consent was obtained from all participants before the start of the study, and they were fully informed about the study objectives and methodology. Moreover, the participants were ensured of the confidentiality of their information, and they were allowed to withdraw from the study at any time.

Results

The majority of the menopausal women (56.7%) and their husbands (37.9%) had less than diploma education. Most of women (60%) had three and more normal deliveries. Other characteristics of the participants are listed in Table 1. The test results showed that the mean score of each subjective symptom and the total score decreased after the intervention, so that the mean total score of subjective symptoms of vaginal atrophy before the intervention was 174.67±35.62; after the intervention, it decreased to 82.83±15.06 in week 2 and 58.83±11.93 in week 4. Moreover, significant differences were observed between before the intervention, two weeks after the intervention, and four weeks after the intervention (P<0.001) (Table 2).

Table 1: Characteristics of the participants

Variable	Mean±SD	Min	Max
Women age (Year)	52.89±5.21	46	65
Husbands age (Year)	56.76±4.91	46	70
Body mass index (kg/m^2)	27.47±2.72	20.31	29.83
Menarch age (Year)	12.67±1.49	11	16
Age of menopause (Year)	48.43±3.74	42	57
Duration in menopause (year)	4.25±3.74	1	15
Number of sexual activities per month	3.62±1.52	1	8
Participants' job	N (%)		
Employed	3 (10)	-	-
Housewife	27 (90)	-	-
Monthly income			
Less than enough	3 (10.0)	-	-
Enough	26 (86.6)	-	-
More than enough	1 (3.4)	-	-

Table 2: Comparison of the mean of each and total score of subjective symptoms of vaginal atrophy in postmenopausal women before and after the intervention

Variable	Before intervention (T1) Mean±SD	2 weeks after intervention (T2) Mean±SD	4 weeks after intervention (T3) Mean±SD	P value*	Pairwise comparison (P value)**
Vaginal burning	16.0±14.99	7.17±9.97	3.33±7.11	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Vaginal itching	6.67±8.84	2.0±4.06	0.33±1.82	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Vaginal dryness	78.17±16.37	33.16±3.59	25.33±4.53	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Dysparunia	71.0±12.34	39.83±6.88	29.83±5.79	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Total score	174.67±35.62	82.83±15.06	58.83±11.93	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)

*Repeated Measure ANOVA test; **LSD post-hoc test

Table 3: Comparison of the mean of each and total scores of descriptive evaluation of vaginal mucosa in postmenopausal women before and after the intervention

Variable	Before intervention (T1) Mean±SD	2 weeks after intervention (T2) Mean±SD	4 weeks after intervention (T3) Mean±SD	P value*	Pairwise comparison (P value)**
Color	2.03±0.41	1.5±0.57	1.0±0.37	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Rugae	1.33±0.6	1.20±0.48	1.16±0.46	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Petechia	0.87±0.86	0.67±0.71	0.51±0.57	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Elasticity	1.42±0.56	1.33±0.47	1.06±0.36	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Dryness	2.10±0.48	1.16±0.37	0.93±0.36	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Total Score	7.70±2.01	5.83±1.31	4.70±1.17	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)

*Repeated Measure ANOVA test; **LSD post-hoc test

Based on the test results, the mean score of each item of descriptive evaluation of vaginal mucosa and the total score decreased after the intervention, so that the mean total score of descriptive evaluation of vaginal mucosa before the intervention was 7.70±2.01; after the intervention, it decreased to 5.83±1.31 in week 2 and 4.70±1.17 in week 4. Moreover, there was a significant difference in terms of these scores before the intervention, two weeks after the intervention, and four weeks after the intervention (P<0.001) (Table 3).

The analysis of variance (ANOVA) showed that the vaginal pH score decreased after the intervention, so that the vaginal pH score before the intervention was 7.70±2.01,

Table 4: Comparison of mean of vaginal pH, vaginal maturation index and value in postmenopausal women before and after the intervention

Variable	Before intervention (T1) Mean±SD	2 weeks after intervention (T2) Mean±SD	4 weeks after intervention (T3) Mean±SD	P value*	Pairwise comparison (P value)**
Vaginal maturation index (%)					
Superficial (%)	24.36±8.24	37.53±6.90	46.63±12.17	<0.001	T1<T3 (<0.001) T1<T2 (<0.001) T2<T3 (<0.001)
Intermediate (%)	46.39±9.69	47.86±8.46	45.70±10.26	0.51	---[a]
Parabasal (%)	28.70±11.40	15.36±10.32	8.73±5.97	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)
Vaginal maturation value	47.51±8.68	61.46±6.79	68.15±7.49	<0.001	T1<T3 (<0.001) T1<T2 (<0.001) T2<T3 (0.002)
Vaginal pH	7.70±2.01	5.83±1.31	4.70±1.17	<0.001	T3<T1 (<0.001) T2<T1 (<0.001) T3<T2 (<0.001)

*Repeated Measure ANOVA test; **LSD post-hoc test; [a]Due to non-significance, the post-hoc test was not performed

and after the intervention it decreased to 5.83±1.31 in week 2 and 4.70±1.17 in week 4. LSD post-hoc test showed that there was a significant difference in terms of vaginal pH score before the intervention, two weeks after the intervention, and four weeks after the intervention (P<0.001) (Table 4).

Regarding the mean percentage of vaginal cells (vaginal maturation index), the percentage of superficial cells increased and the percentage of parabasal cells decreased after the intervention; also, there was a significant difference in terms of vaginal maturation index among the previous stages (before the intervention), two weeks after the intervention, and four weeks after the intervention (P<0.001). VMV also increased after the intervention and a significant difference was observed among different times, i.e. before the intervention, two weeks after the intervention, and four weeks after the intervention (P<0.05) (Table 4).

Regarding the level of satisfaction of postmenopausal women with the use of citrus aurantium vaginal cream, 18 subjects (60%,) 10 (33.33%), 1 (3.34%), and 1 person (3.34%) were reported to be very satisfied, satisfied, neither satisfied nor dissatisfied, and dissatisfied, respectively, at the end of the study. None of the mothers reported any adverse effects due to the consumption of citrus aurantium vaginal cream.

Discussion

The results of this study showed that the citrus aurantium vaginal cream had positive effects on reducing the subjective symptoms of vaginal atrophy, including vaginal burning, vaginal itching, vaginal dryness, and dyspareunia in the second and fourth weeks after the intervention. Regarding the mechanism of action of citrus aurantium, it can be said that this plant is a phytoestrogen that contains flavonoids. The flavonoids can be divided into four groups, including flavanones (main flavonoids), flavones (the second major group of flavonoids), flavonols (in small amounts), and anthocyanins (only in blood oranges).[12] Therefore, the citrus aurantium plant plays a role in regenerating vaginal tissue and collagen production in the same mechanism as estrogens due to its phytoestrogens content which makes it effective in treating vaginal atrophy in postmenopausal women.

The results of a study showed a significant improvement in the subjective symptoms of vaginal atrophy after using chamomile vaginal gel.[21] The results of this study are in

line with those of our study. The flavonoids epigenin and chrysin in chamomile extract have phytoestrogenic effects.[21] Citrus aurantium also contains flavonoids. Based on the results of a study, the subjective symptoms of vaginal atrophy were significantly reduced after using licorice vaginal cream. This study supported our result, showing that licorice contains flavonoids, including isoflavone.[10] A study showed that taking Pueraria Mirifica vaginal gel reduced the subjective symptoms of vaginal atrophy.[22] Citrus aurantium and Pueraria mirifica are both good sources of phytoestrogens. The main components of Pueraria mirifica extract are mirosterol and deoxymirosterol, which have the same molecular structure as estradiol, the strongest form of estrogen.[22] In a study, the researchers concluded that the combined use of oral and vaginal isoflavones versus oral consumption alone improved vaginal dystrophy symptoms, which is consistent with the results of the present study and supports vaginal use of phytoestrogens.[23] According to a study in Iran, vaginal cream containing herbal extracts (Fennel, Salvia officinalis, and Flaxseed) with phytoestrogen effects affected reducing subjective symptoms of atrophy in menopausal women.[24] Fennel is rich in phytoestrogens including lignans and isoflavones.[9] Lignans are also one of the most known phytoestrogens found in flaxseed.[9] The results of another study showed that Nettle vaginal cream reduced subjective symptoms of vaginal atrophy. Nettle and citrus aurantium both contain flavonoids.[25]

The results of the present study confirmed the findings of the mentioned studies concerning the improvement of the subjective symptoms of vaginal atrophy. Chamomile, Licorice, Fennel, Pueraria Mirifica, Isoflavone extract, Salvia officinalis, Flaxseed, and Nettle all contain phytoestrogens. Phytoestrogens have a positive effect on the function of the vaginal mucosa with the ability to bind to beta estrogen receptors.[9]

Moreover, citrus aurantium vaginal cream improved the descriptive evaluation of vaginal mucosa including color, rugae, petechiae, elasticity, and vaginal dryness in the second and fourth weeks after the intervention compared to before the intervention, and this difference was statistically significant. This finding confirms the positive effect of phytoestrogens on vaginal mucosal function.[9]

The decreased level of circulating estrogen, associated with normal aging and menopause, leads to the breakdown of collagen and elastin fibers in the vagina and results in the atrophy of the vaginal epithelium, pale and thin epithelium, decreased vaginal elasticity, loss of rugae, vaginal shortening and narrowing, decreased vaginal blood flow, and loss of vaginal moisture in response to sexual arousal.[26, 27]

The results of this study also showed that the citrus aurantium vaginal cream had a positive effect on lowering the vaginal pH in the second and fourth weeks after the intervention compared to before the intervention, and this difference was statistically significant. The results of studies on the effect of fennel and licorice vaginal cream on postmenopausal vaginal atrophy showed that vaginal pH decreased following the consumption of these herbs.[10, 28] These results were consistent with the findings of the present study regarding the improvement of vaginal pH and can be explained by the fact that citrus aurantium, fennel, and licorice contain phytoestrogens, which are like natural female estrogens in terms of structure and function.[9]

The results of the present study also showed that citrus aurantium vaginal cream significantly increased the superfacial cells two and four weeks after the intervention. Moreover, the VMV improved significantly after the application of citrus aurantium vaginal cream compared to before the intervention. The results of a study indicated that the superfacial cells increased in the fennel vaginal cream group compared to the control group; however, the parabasal and middle cells decreased compared to the controls.[28] A study reported that the degree of maturity of the vaginal cells increased significantly after consumption of Pueraria Mirifica vaginal gel. Pueraria Mirifica causes

estrogenic effects on the vaginal tissue and can reduce symptoms of vaginal epithelium atrophy in postmenopausal women.[22] In another study, daily consumption of isoflavone vaginal gel was associated with a significant increase in the maturation degree of vaginal cells in postmenopausal women.[29] Isoflavones, polyphenol flavonoids, contain a phenolic ring in the same position as estradiol, which allows isoflavone receptors to occupy different tissues and have similar actions to estrogen. Isoflavones also cause a significant increase in the number of blood vessels in vaginal tissue.[30] According to the results of a study, licorice could increase the VMI. This elevation of VMI may be because licorice has isoflavones as herbal estrogens.[10] These results are consistent with those of the present study concerning the improvement of vaginal cell maturation.

One of the strengths of this study is that the effectiveness of Citrus aurantium vaginal cream has been investigated by using several tools and evaluating several parameters. Regarding the limitations of this study, one can refer to limited access to the study samples which made it impossible to conduct a clinical trial on the control group. Not using a placebo was another limitation of the study. Moreover, participants were selected from the clients referred to the clinic. All these limitations may have compromised the generalizability of the results.

Conclusion

Based on the obtained results, citrus aurantium vaginal cream improved subjective symptoms of vaginal atrophy, reduced the score of descriptive evaluation of vaginal mucosa, decreased vaginal pH, and increased vaginal maturity in postmenopausal women. Further studies with similar methodologies, larger sample sizes, and a control group are recommended to confirm the findings of this study.

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