





Effectiveness of a Herbal Vaginal Tablet Containing Oak Gall and Ajwain on Vaginitis: A Randomized, Double-blind Pilot Study

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Abstract

Background and objectives: Vaginitis is one of the most common diseases in women. Oak gall and ajwain have been used in traditional Persian medicine for treatment of vaginitis. The purpose of this study was to formulate a vaginal preparation containing oak gall and ajwain and evaluate its effectiveness on the recovery and recurrence of vaginitis. **Methods:** The present pilot study is a randomized, double-blind clinical trial performed on 24 women with mixed vaginitis, including bacterial vaginitis and trichomoniasis in a gynecology center. Subjects were divided into two groups receiving herbal vaginal tablets or metronidazole vaginal tablets for 7 days. The vaginal tablets were prepared using dried extract of oak gall and essential oil from ajwain by direct compression method. Clinical signs and laboratory tests were assessed after treatment. The symptoms were evaluated on day 10, and also 4 and 12 weeks after intervention. **Results:** There was a statistically significant difference in sexual function, and characteristics of secretions including amount, pH, odor, leukocyte count and parasite content in both groups of herbal (oak gall and ajwain) and metronidazole vaginal tablets before and after treatment ($p < 0.05$). The group receiving herbal vaginal tablets showed significantly reduced secretion at follow-up on day 10 and after 4 weeks ($p < 0.05$).

Conclusion: In our pilot study, herbal vaginal tablets containing oak gall and ajwain were as effective as metronidazole vaginal tablet. The results provide a good basis for future confirmatory tests.

Keywords: bacterial vaginitis; Persian medicine; *Quercus infectoria*; *Trachyspermum ammi*; *Trichomonas vaginitis*

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Introduction

Vaginitis is one of the most common genital infections, with 75% of women experiencing it at least once in their lifetime [1]. The prevalence of vaginitis in different populations has been

reported as 11-48% [2]. Approximately, 90% of vaginitis cases are caused by infectious agents such as bacteria (50-40%), *Candida* (25-20%) and *Trichomonas vaginalis* (15-15%) [3]. In

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addition to the high incidence of this infection, physicians face challenges in treatment, including non-response to treatment, drug resistance, drug side effects, and frequent recurrence in about 70% of cases [4].

Vaginitis, especially that caused by bacteria, predisposes individuals to pelvic inflammatory disease, preterm labor, miscarriage and infertility; thus, early diagnosis and treatment is necessary to prevent complications [5]. Medical history combined with clinical examination is the simplest method of diagnosis, with findings including dyspareunia, dysuria, vaginal discharge, itching, unpleasant odor and redness of the vulva [6].

The most routine treatments include metronidazole or clindamycin in bacterial vaginitis, metronidazole in trichomoniasis infection, and clotrimazole or fluconazole in *Candida* infections [6]. The response rate to metronidazole in bacterial infections is about 70-80%, and vaginal use is more widely accepted due to less systemic side effects. Oral metronidazole is still the treatment of choice for trichomoniasis vaginitis [7].

Decreased effectiveness of existing treatments, increased duration of treatment, emergence of new and resistant strains, high recurrence rates, drug side effects, and high costs of medical treatments indicate the need for research on new treatment options such as herbal drugs [8-10].

Ajwain, an herbaceous plant scientifically named *Trachyspermum ammi* (L.), belongs to Umbelliferae family [11]. The essential oil content of the fruit is between 2.4-2.8% and its main constituents include thymol, *p*-cymene and γ -terpinenol [12].

According to traditional Persian medicine, ajwain has been used in the treatment of various uterine diseases. To cleanse the uterus and eliminate foul-smelling moistures of the genital tract, topical use in the form of vaginal fumigation, vaginal tablets and douches has been recommended [13,14]. In vivo studies have demonstrated that aqueous and ethanolic extracts of ajwain have anti-inflammatory, antibacterial and antifungal properties [15]. Moreover, ajwain essential oil has demonstrated effectiveness on vaginal pathogens invitro [16].

Quercus infectoria G. Olivier belongs to the Fagaceae family [17]. The gall of this plant tonifies the uterus, eliminates excessive uterine secretions, and is used to treat intestinal ulcers

and chronic diarrhea according to traditional Persian medicine [13, 18]. Oak gall has revealed activities against pathogens involved in vaginitis including bacterial, fungi, and *Trichomonas vaginalis* [19].

Due to the overlap of symptoms of bacterial and trichomoniasis vaginitis and the high percentage of mixed infections, this study was designed to investigate the effect of the prepared herbal product (vaginal tablets containing ajwain and oak gall) on management and recurrence of bacterial and trichomoniasis vaginitis.

Material and Methods

Ethical considerations

This pilot study was a double-blind randomized clinical trial, performed according to the declaration of Helsinki, and approved by the ethics committee of Shahid Beheshti University of Medical Sciences on 2017-10-22 (IR.SBMU.RETECH.REC.1396.463), and was registered at the Iranian Registry of Clinical Trials (registration code: IRCT20180217038764N2).

Drug formulation

Ajwain fruit and oak gall were purchased from Iranian herbal market. Oak gall was extracted with 70% ethanol by maceration method for 10 days (1:10), concentrated by a rotary evaporator, and dried by a vacuum oven. Ajwain essential oil was prepared via hydro distillation using a Clevenger apparatus. The vaginal tablets were made using dried extract of oak gall (10%) and essential oil from ajwain (2%) by direct compression method. The amount of thymol and gallic acid were determined [20]. For microbial control of the product, total aerobic microorganisms, total molds and yeasts, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were determined on the final product [21].

Pilot study

Sample size

The sample size in this pilot study was considered 16 people in each group.

Inclusion and exclusion criteria

Thirty-two women aged 18-40 years, and diagnosed with bacterial or trichomoniasis vaginitis or both, based on clinical examination and laboratory criteria, referring to Anahita Midwifery Counseling and Services Center in Ahvaz, entered the study in 2017, after providing

informed consent. The patients were screened by the researcher.

Pregnant and lactating participants, those with recent drug use (antiparasitic, antibiotics, immunosuppressive drugs), sexual intercourse and vaginal douching during the last 48 hours, abnormal uterine bleeding, certain diseases such as liver disease, systemic diseases, central nervous system disorders, blood dyscrasias, diabetes, immune deficiency, sexually transmitted diseases, and sensitivity to drug components were excluded.

Intervention

Patients allocated to two groups (herbal group and metronidazole group) using a permuted block randomization with four size blocks and a random number table (all subjects were randomized using a random number table that was generated as a set of numbers in a specific order to read the numbers from left to right. The herbal group received the herbal vaginal tablet containing oak gall and ajwain, while the metronidazole group received vaginal metronidazole tablet for 7 days.

Outcomes

Demographic information of patients was collected. According to Persian medicine, uterine temperament is involved in susceptibility to uterine diseases such as vaginitis, thus the patients' uterine temperament was recorded using a uterine temperament questionnaire [22]. Vaginal discharge was evaluated and compared before and after treatment in terms of amount, pH, Whiff test, clue-cells, leukocyte count, and number of flagella.

The sample of vaginal secretions was collected for microscopic examination and clue-cell detection in fresh smear. One sample was used for Nitrazine test to determine pH. Saline wet mount test was done for diagnosis of trichomonas infection. Whiff test was done by adding a drop of 10% potassium hydroxide solution to a sample of vaginal discharge.

The Iranian version of the Female Sexual Function Questionnaire (FSFI) (Rosen et al. with a Cronbach's alpha of 0.86 and ICC correlation coefficient of 0.77) was evaluated before and after treatment in the two groups [23]. To assess the recurrence of symptoms, complaints including the presence of discharge, burning, itching, and discharge characteristics including color, odor and consistency were evaluated ten

days, 4 weeks and 12 weeks after treatment and compared in the two groups. Participants were observed for any drug side effects.

Statistical analysis

To analyze clinical and para clinical data of participants, Friedman test and independent t-test were used to compare the two groups at the beginning of the study. Comparison of the results of the two groups before treatment, and at weeks one, four, and twelve after treatment was done using Friedman test. All analyses were performed with SPSS software version 23 and $p < 0.05$ was considered significant.

Results and Discussion

All the tests performed on herbal vaginal tablets were in the normal range. Mean of gallic acid and thymol content in each herbal vaginal tablet were 14.27 ± 0.5 mg and 9.91 ± 0.1 mg, respectively.

In accordance with United States Pharmacopeia (USP) guidelines [21], the total microorganism and fungal count were in the normal range and there were no *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* in the herbal vaginal tablets.

Considering the inclusion criteria, thirty-two patients with bacterial, trichomoniasis or mixed vaginitis were included in the study and divided into two groups. Four participants in each group were excluded (personal reasons in metronidazole group, and drug side effects include burning and itching in herbal group), and thus each group was analyzed based on the results obtained from the twelve remaining participants. Figure shows the flowchart of the studied patients.

The age of participants lied in a range between 18 to 40 years (mean = 29.45 ± 7.14). Regarding demographic data, there was no significant difference between the two groups in terms of age, education or occupation. Uterine temperament was cold and wet in the majority of patients. The demographic status and frequency of symptoms at the beginning of the study are demonstrated in Table 1.

Vaginal discharge was the most common symptom in the evaluation of clinical and laboratory symptoms experienced by 23 patients (95.9%) at the beginning of the study.

The pH of vaginal discharge was 4.5-5.5 in most patients. Whiff test, clue-cells and leukocyte count decreased with treatment. The frequency of clinical and laboratory symptoms before and after

treatment was not significantly different ($p>0.05$) between the two groups. Clinical and laboratory findings of each group at the beginning and the end of the study were compared. Intervention resulted in significant reduction of all clinical and laboratory symptoms in both groups, except for the number of clue-cells. Frequency of clinical and laboratory symptoms before and after intervention are demonstrated in Table 2.

In both groups, the score of sexual activity increased significantly with treatment. A comparison of sexual function in the two groups before and after treatment is provided in Table 3. To evaluate recurrence, vaginal examination was performed in all patients 10 days, 4 weeks and 12 weeks following the treatment. At day 10 and week 4, the number of patients with no complaint were significantly higher, and the amount of foul-smelling secretion was significantly lower in the herbal group ($n=0$) compared to the metronidazole group ($n=7$). Also, the amount of foul-smelling discharge in the herbal group was

significantly lower than the metronidazole group 12 weeks after treatment. A comparison of symptoms in the two groups on day 10, week 4 and week 12 post-treatment is illustrated in Table 4.

Three participants complained of abdominal pain at the time of use. Two other patients reported genital burning upon drug administration. Regarding herbal tablets containing oak gall and ajwain, slow dissolution of the herbal tablets was reported by three participants.

In the present study formulation of vaginal tablets containing ajwain essential oil and oak gall extract and performing the relevant physicochemical tests was done for the first time. The mean age of participants in this trial was 29.45 ± 7.14 . In Masoudi et al. study on bacterial vaginosis, the average age was about 32.88 ± 6.04 [24], which is comparable with the present study, regarding the high prevalence of vaginitis in sexually active women of reproductive age [25].

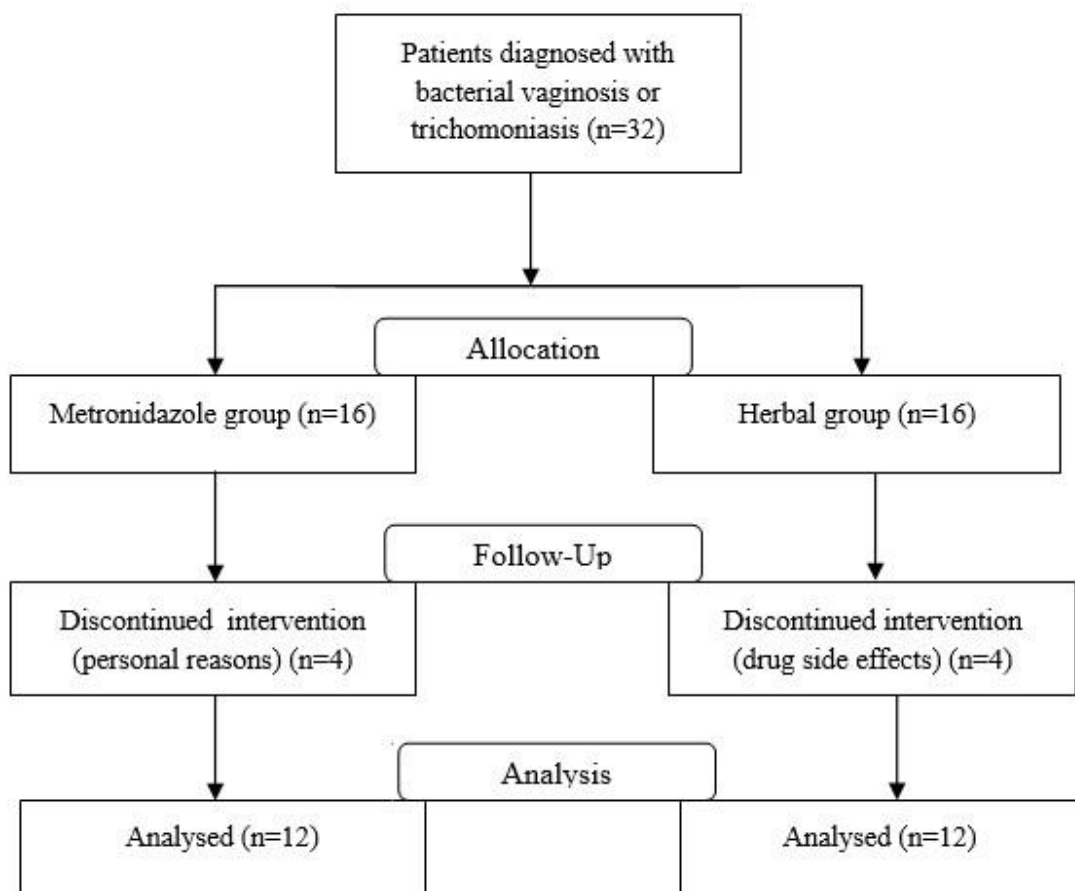


Figure 1. Consort flow diagram

Table 1. Demographic status and frequency of symptoms at the beginning of the study

Variable	Herbal group	Metronidazole group	p-Value
Age (mean ± SD)	28.58±7.46	30.33±7.02	0.560
Education (n)	Below high school diploma	1	0.482
	High school diploma	7	
	Bachelor degree or higher	4	
Employment (n)	Employed	6	1.000
	Housewife	6	
Uterine temperament (n)	Cold	7	1.000
	Hot	5	
	Wet	8	0.640
	Dry	4	

Table 2. Frequency of clinical and laboratory symptoms of bacterial and *Trichomonas* vaginitis before and after intervention

	Pre-treatment			Post-treatment		
	Herbal group (N=12)	Metronidazole group (N=12)	p-Value	Herbal group (N=12)	Metronidazole group (N=12)	p-Value
Vaginal discharge	Yes	12	0.307	10	12	0.140
	No	0		2	0	
	Pre/post-treatment comparison in herbal group			Pre/post-treatment comparison in metronidazole group		
pH of secretions	<4.5	1	0.537	7	4	0.219
	>4.5	11		5	8	
	Pre/post-treatment comparison in herbal group			Pre/post-treatment comparison in metronidazole group		
Whiff test	Positive	12	0.064	0	0	0.064
	Negative	0		12	12	
	Pre/post-treatment comparison in herbal group			Pre/post-treatment comparison in metronidazole group		
Clue-cells	Yes	7	0.098	0	0	-
	No	5		12	12	
	Pre/post-treatment comparison in herbal group			Pre/post-treatment comparison in metronidazole group		
Leukocyte	Yes	11	1.00	4	5	0.673
	No	1		8	7	
	Pre/post-treatment comparison in herbal group			Before/after comparison in metronidazole group		
Flagella	Yes	11	0.158	2		0.537
	No	1		10	11	
	Pre/post-treatment comparison in herbal group			Before/after comparison in metronidazole group		

Results of the present study demonstrated a significant difference between the amount of secretion before and after treatment in both herbal and metronidazole groups, which indicates the effectiveness of both drugs in reducing discharge. In a study on bacterial vaginitis, the amount of discharge was significantly reduced to 15.8% in the metronidazole group. Also, in the group receiving herbal suppositories (containing myrtle, tribulus, fennel and tamarind), 79.6% had no discharge and 15.6% showed minimal discharge at the end of intervention, while 4.6% still complained of excessive discharge [26]. In the study by Ahmad et al., oral administration of a herbal formula containing oak gall improved itching, burning, vaginal discharge and back pain

by 86.66%, while the metronidazole group experienced 93.33% improvement [27].

In the present study, there was a significant difference between pH of secretions before and after both herbal and metronidazole groups, while no significant difference was observed between the two groups. A pH less than 4.5 (between 3.8 to 4.2), inhibits the growth of microorganisms and is observed in healthy women throughout the menstrual cycle. A change in this variable indicates imbalance in the vaginal environment, resulting in decreased lactobacilli and increase in other microorganisms. These conditions predispose the individual to bacterial vaginosis [28].

Table 3. Comparison of sexual function in metronidazole and herbal groups before and after treatment

		Pre-treatment	Post-treatment	p-Value
Sexual function	Herbal group	19.09±2.98	25.00±3.02	0.000
	Metronidazole group	19.33±3.62	25.97±4.77	0.000
p-Value		0.860	0.560	

Table 4. Comparison of symptoms in the two groups on day 10, week 4 and week 12 post-treatment

		10 Days post-treatment		4 Weeks post-treatment		12 Weeks post-treatment	
		Herbal group	Metronidazole group	Herbal group	Metronidazole group	Herbal group	Metronidazole group
Complaint	No complaint	8	2	6	2	3	1
	Foul-smelling discharge	0	7	0	7	1	6
	Burning	3	2	1	3	3	4
	Itching	0	1	1	0	2	1
p-value		p=0.008		p=0.014		p=0.194	
Color of discharge	Colorless	9	6	7	4	4	1
	Colored	3	6	5	8	8	11
p-Value		p=0.206		p=0.414		p=0.317	
Consistency of discharge	Thick	0	0	3	2	5	2
	Thin	12	12	9	10	7	10
p-Value		-		p=1.000		p=0.397	

Jafarzadeh et al. investigated the effect of lactobacillus and metronidazole suppositories in vaginal PH. Their results were indicative of significant change in both groups [29]. In a study by Rabiei et al., vaginal pH was not significantly different between two groups receiving either tinidazole or metronidazole [4]. Whiff test was positive at the beginning of treatment in 21 patients (87.5%), but negative in all participants after treatment. Results showed that there was a significant difference in the disappearance of fishy odor before and after treatment with both herbal and metronidazole suppositories. In a study by Khan, administration of a herbal powder ("GuleD hawa" and "Mocharas") reduced the positive Whiff test in bacterial vaginitis from 26 patients (86%) at the beginning of the intervention to 8 patients (31.8%) after treatment [30].

In the present study, there was a significant difference between the number of clue-cells before and after herbal treatment, while no significant difference was observed in the metronidazole group. This indicates a decrease in the number of bacteria with herbal treatment. The study by Simose et al. reported that 77% of participants still had clue-cells in secretions after one week of treatment with metronidazole gel [31]. In a study by Baery, a significant decrease in clue-cells was observed with treatment in both groups of herbal tablets (containing *Tribulus*

terrestris, *Myrtus commuis*, *Foeniculum vulgare* and *Tamarindus indica*) and metronidazole [26].

In the present study, there was significant difference between leukocyte count, and parasite count before and after herbal treatment. There was also significant difference in leukocyte count, and parasites before and after treatment with metronidazole.

To evaluate recurrence, vaginal examination was performed at day 10, week 4, and week 12 after completion of intervention. Considering the fact that vaginal discharge is one of the most common and important symptom in patients with vaginitis [6], improvement of this complaint during the follow-up was regarded as an indicator of non-recurrence. A significantly greater reduction in vaginal discharge on day 10 and week 4, and in discharge odor at week 12 was found in the herbal group compared to the metronidazole group. Marcone et al. reported that in a quarterly follow-up of patients prescribed with a weekly dose of lactobacilli vaginal suppositories for two months after one week of oral metronidazole treatment, 88% were negative for bacterial infection [32].

In the present study, herbal and metronidazole tablets both improved female sexual function. There was no significant difference in the effect of these two medications in improving sexual function. Foul-smelling discharge and vaginal burning are causes of sexual dysfunction, which

were alleviated by both drugs. This resulted in improved sexual function and therefore had a positive impact on quality of life of patients. A study by Bradshaw showed a link between bacterial vaginosis and reduced frequency of sexual activity [33].

The causes of vaginitis, as described in Persian medicine, comprise local causes such as infection and uterine weakness, as well as systemic etiologies including predominance of the humors (blood, phlegm, yellow bile or black bile). Accordingly, trichomoniasis vaginitis is associated with predominance of yellow bile, since it causes foul-smelling and yellowish secretions. In contrast, opaque and gray discharge in bacterial vaginitis is consistent with the predominance of black bile [34]. From the perspective of PM, the three imperative principles in the treatment of vaginitis include uterine cleansing, body cleansing and uterine tonification and strengthening. Uterine cleansing is mainly achieved by topical drug administration via drugs with constrictive and uterine cleansing actions [35,36]. One of the major challenges in the treatment of vaginitis, is recurrence of the signs and symptoms. PM scholars emphasized on tonifying the uterus after treatment, in order to reduce susceptibility to infections and thus recurrence. Complete cleansing the body of excess humors is also considered as a proper method to prevent recurrence of infection [34].

According to PM resources, ajwain has a hot and dry temperament, and vaginal application is used to cleanse the uterus from foul-smelling moistures [13]. This herb has also been used for enhancing sexual power, and regulation of menstruation [15] and has been recommended for the treatment of vaginal discharge [37]. Studies have demonstrated that ajwain has bactericidal and bacteriostatic properties, related to phenolic compounds such as thymol. Anti-inflammatory and antioxidant effects of this herb have also been proven [16]. Ajwain is also known to be effective in the treatment of various types of vaginitis due to antimicrobial, anti-candida and anti-*Trichomonas vaginalis* effects [16]. Oak gall has a cold and dry temperament and is a powerful constrictive agent, and is thus used to remove vaginal moistures and strengthen the uterus [13]. This herb contains gallic acid and can restore the elasticity of the uterus after delivery and stimulates contraction of vaginal muscles [38]. Oak gall regulates macrophage and neutrophil functions and inhibits inflammatory mediators

such as PGE₂, NO, O₂, and lytic enzymes [39]. The tannins in oak gall, which is reported to be approximately 50-70%, are responsible for many of the plant's anti-inflammatory, antibacterial and antifungal properties [38]. It is used in the treatment of ulcerative colitis, rectal abscess, gingivitis, oral aphthous and ulcers [40,41]. The effects of oak gall against vaginitis pathogens such as bacteria, *Trichomonas* and *Candida* has been demonstrated in studies [19]. The anti-inflammatory, antibacterial and antifungal properties of this herbal material are related to flavonoids, alkaloids, phenols, and especially tannins [42,43]. Studies have also reported hepatoprotective effects [44] and immune system regulation properties [45].

Conclusion

In this study, administering oak gall and ajwain vaginal tablets for one week significantly reduced vaginal discharge and other symptoms of bacterial and trichomonas infections and improved sexual function in participants. Further large clinical trials are necessary for evaluation of the efficacy and safety of this drug.

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Author contributions

Mozhgan Mehri Ardestani, Roja Rahimi and Mojgan Tansaz developed the original idea; Mozhgan Mehri Ardestani, Afrooz Mirahi and Tayebeh Toliyat carried out the experiments; Maryam Bahman took the lead in writing the manuscript. All authors provided critical feedback and helped in the research, analysis and writing the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

GC-MS: gas chromatography–mass spectrometry; ICC: intra class correlation coefficient; NO: nitric oxide; PGE2: prostaglandin E2; PM: Persian medicine; USP: United States Pharmacopeia