Comparison of the Effects of Artemisia Vaginal Cream and Clotrimazole on Vaginal Candidiasis Infection

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SUMMARY

*Candida* vaginitis is the second most common vaginal infection which is treatable by azole. Over-consumption of azole is associated with resistance to drug and treatment failure. Studies conducted in laboratory conditions confirm the anti-fungal effects of Artemisia plant. The aim of this study was to compare the effect of vaginal Artemisia cream and clotrimazole on vaginal candidiasis infection.

This is a clinical triple-blind trial that was performed on 72 non-pregnant women referred to Mashhad health centers in 2012-2017. After confirmation of *Candida* infection, the research units were randomly allocated into two groups: Artemisia and clotrimazole vaginal cream recipients treated for 7 nights. After the treatment, the data were analyzed by SPSS software (version 24).

The results of the study showed that the mean reduction in the score of signs and symptoms of vaginal *Candida* infection was 7.4 ± 4.1 and 7.6 ± 3.8 in the Artemisia and clotrimazole group, respectively. Wilcoxon test showed that improvement in both groups treated by Artemisia and clotrimazole was significantly higher than before treatment (p < 0.001). There was no significant difference between the two groups regarding the improvement of symptoms (p > 0.05).
The present study showed that Artemisia vaginal cream and clotrimazole cream both improved the symptoms related to vaginal Candida infection. In addition, the therapeutic effect of the two drugs was not statistically significant in comparison with each other. Therefore, it can be used as an alternative treatment for vaginal candidiasis.

Key words: Candida vulvovaginitis, Artemisia, clotrimazole

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INTRODUCTION

Vaginitis is one of the common diseases caused by the genital yeasts. The three main kinds of vaginitis are bacterial vaginosis (BV), vaginal candidiasis, and trichomoniases (1). Candida infection is the second most common type of vaginal infection after bacterial vaginosis (2), usually caused by Candida albicans (3). This microorganism is found in the reproductive system of 30-80% of women, being asymptomatic or giving mild symptoms (2). About 75% of women experience Candida vulvovaginitis at least once in their lifetime, and 45% of women have two or more infections yearly (1). Some women also experience chronic and recurrent infections. The prevalence of this disease has been reported from 12.5% to 44% in the world (4-6) and from 16.5% to 35.5% in Iran (7-10). The factors that have been claimed to contribute to the development of Candida infections include: immunosuppressive drugs (11), antibiotics (12) and diabetes (1, 2).

The symptoms of Candida include itching, burning, or both of these symptoms. Itching may be severe and it may be felt in the vagina or the vulva. Irritation is accompanied by irritation from the urine or painful intercourse. Candida infection secretion is often thick and white like cottage cheese or thin and watery (2). If there is suspicion of Candida vulvovaginitis based on the clinical symptoms, the diagnosis is confirmed by direct testing using Nickerson or Saboraud Dextrose Agar (2). There are several options for the treatment of vaginal candidiasis (2, 13). Azole is one of the drugs used to treat vaginal candidiasis. These drugs cause other microorganisms to grow by changing the vaginal flora. It could be also associated with complications such as vaginal burning and irritation, rash, abdominal pain and cramping. Excessive consumption of azole causes resistance to the drug, which results in failure of treatment and recurrence of the disease (14, 15). Therefore, the use of products with antifungal effects and lower complications seems necessary. One of the main therapeutic methods is herbal medicine, which has been considered in recent decades due to less complications and more adaptation of patients to these drugs (15). In traditional medicine, some plants such as Artemisia is known as antifungal plants (16-18). Artemisia belongs to the Cichorium family and with more than 34 species has been known as the main and most herbal element of the pastures in Iran (19). During research on 240 species of Cichorium to determine their medicinal properties, about 84 compounds have been identified in Artemisia species (20, 21). It has been introduced as anti-inflammatory (22, 23), antifungal (17, 22), antibacterial (24), antiviral (25, 26), antioxidant (17, 22, 27), anti-microbial (28, 29), anti-diabetes (22, 30), and immune booster plant (31, 32) in traditional medicine books and some studies. This study was conducted to compare the effect of Artemisia vaginal cream and Clotrimazole on vaginal candidiasis infection, considering the antifungal effects of Artemisia, especially against resistant species to chemical treatments such as Candida glabrata. It was also used because of its anti-inflammatory properties in the laboratory environment (15, 33), due to the high incidence of vaginal candidiasis, unpleasant side effects of azole (34) and resistance to the drug (27). In addition it was used because of the need for an effective drug with less complications and also women’s desire to use more herbal drugs.

MATERIAL AND METHODS

Participants and Procedures

This clinical triple-blind trials study was conducted after the consent of the ethics committee. This research was done on all non-pregnant women in their reproductive age, referred to the health centers Tajir Abad and Baze Sheikh in Mashhad in 2016-2017. These centers were selected due to the high number of referrals for examination. The sample size was included 36 participants in each group, 72 participants in total; the confidence interval was 95%, power 80%, and the rate was 20%, using the SPSS software. At the beginning of the study, 72 participants entered the study, 7 of them were excluded due to the lack of continuation (two participants because of not using condoms more than once, 5 participants were not referred on time). Therefore, the final analysis was performed on 65 subjects.

The inclusion criteria were as follows:

- Reading and writing literacy;
- Willingness to participate in the study;
- Age range of 15-49 years;
- Being married;
- The absence of any known medical and psychological illness;
- No prior history of susceptibility to Clotrimazole and chicory;
- Non being pregnant or breast-feeding;
- Not using IUD and hormonal contraceptive methods;
- Non-recurrence of Candida vulvovaginitis;
- Not using oral or topical antifungals for the treatment of vaginal infection;
- The minimum earned score of 3 out of 18;
• Symptoms of vaginal candidiasis;
• Positive laboratory results for Candida infection.

The exclusion criteria were as follows:
• Hurricane incident;
• Pregnancy;
• Forgetting to take medication more than once;
• Not using condom more than once, and
• Intolerance or susceptibility to the prescribed drug.

The definitive diagnosis of Candida infection in this study was based on direct test results and vaginal secretion culture. Firstly, all suspected cases of vulvo-vaginitis were interviewed and if they met criteria for entering the study, the examination was performed. In order to confirm the diagnosis, a sample of posterior caudal lobes was taken from the vagina and sent to the laboratory for culture. After a while, the Candida species was determined using the guidance table and the color changes in the culture media. In case the patient was diagnosed with Candida infection, she was entered as a definite sample and randomly assigned to one of the two groups of Artemisia and Clotrimazole. Each group was treated with 1% Clotrimazole cream, manufactured by Abouryhan Pharmacy Company (an Applicator nightly), or 1% Artemisia vaginal cream, manufactured at Mashhad Pharmaceutical Faculty (an Applicator nightly) for one week. The patients were asked to fill the applicator and insert one applicator full of cream into the vagina for a week. In addition to providing complete training to patients in both groups, the use of medications and the observance of health tips were given to them in the form of a pamphlet. Research units were asked to complete the drug registration checklist and possible side effects of the drug after each use and bring it for the next visit. The form of the interview and observation was filled by the researcher following the treatment and after the completion of the vaginal cream (one week ± 24 hours) treatment. If treatment was unsuccessful in Artemisia group, routine treatment was performed. Then, the success rate of treatment was measured and compared in the control and intervention group. In this study, the treatment was considered successful if the candida laboratory test was negative. Information gathering tools included: form of the selection of research units and criteria table, severity of signs and symptoms of Candida infection of the vagina, the form of personal and family characteristics, midwifery and women’s information form, health information and information about Candida infection form, the form of laboratory results before and after treatment and response to treatment based on clinical symptoms, with-drawal form during research, checklist of drug use and symptoms during the treatment. Validity of all forms was evaluated based on the research objectives, the study of the latest sources and related articles, and consultation with the supervisors and counselors. The reliability of the forms was confirmed by the equivalence of reliability (agreement of evaluators r = 90%). After collecting and encoding data, data were analyzed by SPSS software version 24 using descriptive and analytical statistical methods such as Kromogroff-Smirnov test, Kruskal-Wallis test, Mann-Whitney test, Wilcoxon test, Chi-square and Fischer’s exact test. All statistical tests were performed with a confidence interval of 95% and alpha coefficient of 0.05%. The significance level was considered less than 0.05. In this study, the Ethical Code of the Helsinki Declaration were considered.

Data analysis

All data collected through the questionnaire and checklists were statistically analyzed regarding the study aims. These data analyzed using SPSS version 24.

RESULTS

A study on the demographic characteristics of the units showed that the mean age for Artemisia and Clotrimazole group was 32.5 ± 7.1 and was 33.1 ± 7.6 years, respectively. Most of the participants had elementary literacy and were housewives. Significant differences were not observed in terms of age, occupation, education, body mass index, duration of fungal infection, observation of health points, and mean duration of menstruation and symptoms of vaginal disease. Both groups were homogeneous (p > 0.05) (Table 1).

The findings of this study showed that most participants of both Artemisia and Clotrimazole group practiced withdrawal (28.6% in the Artemisia group and 11.8% in the Clotrimazole group; the Chi-square test showed no significant difference between two groups). In this study, there was no a significant relationship between a contraceptive method and infection (p > 0.05).

The results of the study showed that the laboratory test was negative in both Artemisia (66.7%) and Clotrimazole (80.6%) group after treatment (p = 0.99). The Chi-square test did not show significant differences (p = 0.99).

Before treatment, the mean score of signs and symptoms of Candida infection was reported as 13.9 ± 4.0 and 12.7 ± 4.0 in Artemisia and Clotrimazole group,
respectively. Independent T-test did not show significant differences ($p = 0.215$). After treatment, this mean was reported as $6.5 \pm 2.2$ in the Artemisia group and $6.6 \pm 1.6$ in the Clotrimazole group. The Mann-Whitney test did not show significant difference ($p = 0.518$). In addition, in the Artemisia group, the mean reduction in the symptoms of vaginal Candida infection was reported to be $7.4 \pm 1.4$ and $6.3 \pm 3.8$ in the Clotrimazole group. The Mann-Whitney test did not show significant differences ($p = 0.186$). The Wilcoxon test revealed that improvement of the signs and symptoms of vaginal candidiasis was significant in both groups using Artemisia ($p < 0.001$) and Clotrimazole ($P < 0.001$) compared to pre-treatment. There was no statistically significant difference between the two groups in this respect ($p > 0.001$) (Table 2).

### Table 1. Individual and family characteristics of the research units divided into two groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clotrimazole</th>
<th>Artemisia</th>
<th>Intergroup test results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary education and</td>
<td>(58/321)</td>
<td>16 (51/6)</td>
<td>$U = 531/0$</td>
</tr>
<tr>
<td>able to read and write</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>7 (19/4)</td>
<td>8 (25/8)</td>
<td>$P = 0/797$</td>
</tr>
<tr>
<td>Diploma</td>
<td>6 (16/7)</td>
<td>6 (19/4)</td>
<td>Mann-Whitney</td>
</tr>
<tr>
<td>University degree</td>
<td>2 (5/6)</td>
<td>1 (3/2)</td>
<td></td>
</tr>
<tr>
<td><strong>Job</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>3 (94/4)</td>
<td>29 (93/5)</td>
<td></td>
</tr>
<tr>
<td>Employed but working at home</td>
<td>1 (2/8)</td>
<td>1 (3/2)</td>
<td>Chi = 0/0, df = 2</td>
</tr>
<tr>
<td>Employee</td>
<td>1 (2/8)</td>
<td>1 (3/2)</td>
<td>$P = 1/000$</td>
</tr>
<tr>
<td>Student</td>
<td>0 (0/0)</td>
<td>0 (0/0)</td>
<td>Chi square</td>
</tr>
<tr>
<td><strong>Spouse’s education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary and able to read and</td>
<td>1 (38/2)</td>
<td>11 (39/3)</td>
<td>$U = 443/5$</td>
</tr>
<tr>
<td>write</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>14 (41/2)</td>
<td>8 (28/6)</td>
<td>$P = 0/626$</td>
</tr>
<tr>
<td>Diploma</td>
<td>6 (17/6)</td>
<td>7 (25/0)</td>
<td>Mann-Whitney</td>
</tr>
<tr>
<td>University degree</td>
<td>1 (2/9)</td>
<td>2 (7/1)</td>
<td></td>
</tr>
<tr>
<td><strong>Spouse’s job</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worker</td>
<td>14 (41/2)</td>
<td>16 (57/1)</td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>1 (2/9)</td>
<td>0 (0/0)</td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>1 (2/9)</td>
<td>0 (0/0)</td>
<td>$Chi = 2/8, df = 4$</td>
</tr>
<tr>
<td>Self-employee</td>
<td>14 (41/2)</td>
<td>9 (32/1)</td>
<td>$P = 0/762$</td>
</tr>
<tr>
<td>Student</td>
<td>0 (0/0)</td>
<td>0 (0/0)</td>
<td>Chi square</td>
</tr>
<tr>
<td>Others</td>
<td>4 (11/8)</td>
<td>3 (10/7)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Mean and standard deviation of the score of the symptoms of vaginal candidiasis in women before and after treatment by the type of drug

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clotrimazole Mean ± SD</th>
<th>Artemisia Mean ± SD</th>
<th>Intergroup test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>12/7 ± 4/0</td>
<td>13/9 ± 4/0</td>
<td>t = 1/3, df = 64 P = 0/215 Independent T-test</td>
</tr>
<tr>
<td>After treatment</td>
<td>6/6 ± 1/6</td>
<td>6/5 ± 2/2</td>
<td>U = 498/5 P = 0/518 Mann-Whitney</td>
</tr>
<tr>
<td>Difference between before and after treatment</td>
<td>-6/0 ± 3/8</td>
<td>-7/4 ± 4/1</td>
<td>U = 438/0 P = 0/186 Mann-Whitney</td>
</tr>
</tbody>
</table>

Intergroup test result

Z = -5/2 P < 0/001 Wilcoxon test

Z = -4/8 P < 0/001 Wilcoxon test

Before treatment, most of the subjects in the Artemisia group (50%) and in the Clotrimazole group (50%) had mild symptoms of vaginal candidiasis. The Mann-Whitney test did not reveal significant differences (p = 0.366). After treatment, all subjects in the Artemisia and Clotrimazole group had no symptoms of Candida vaginal infection. The Mann-Whitney test did not show a significant difference between the two drugs (p = 1.000).

The improvement of symptoms of vaginal Candida infection after treatment was significant in both groups applying Artemisia (P < 0.001) and Clotrimazole (p < 0.001). Using the Wilcoxon test.

In the field of clinical signs before and after treatment, the Makenmar statistical test showed that in all cases, the reduction of prevalence of symptoms was significant in both groups after treatment. It was not possible to perform a MacNemar test in the Artemisia group, because all participants had symptoms of secretion before treatment. Also, due to the dependence of the results after and before treatment, the Makenmar test was used instead of the Chi square test.

Regarding the fact that itching was the main complaint of vaginal Candida infection in two groups (Artemisia group - 48.4% and Clotrimazole group - 58.3%), the results of this study showed that both drugs decreased itching. Based on the Wilcoxon test, the level of itching in the subjects in the two groups decreased significantly during a week ± 24 hours after the end of the intervention. The level of itching was decreased from the 80% before the intervention to 26.7% (53.3% improvement) after the intervention in the Artemisia group, and from 75% before the intervention to 17.1% (57.9% improvement) after the intervention in the Clotrimazole group (p < 0.001). There was no significant difference between the two drugs according to the Mann-Whitney test (p = 0.326).

Also, the results of this study showed that both drugs reduced other symptoms of candidiasis, such as burning, vaginal irritation and painful intercourse. According to statistical tests, the level of these symptoms in the subjects was significantly reduced during a week ± 24 hours after intervention. In addition, comparison of the effects of two drugs on the reduction of symptoms of vaginal Candida infection was not significant (p > 0.05).

In terms of satisfaction, 100% of patients in the Clotrimazole group and 93.5% in the Artemisia group were satisfied with the treatment method (p = 0.271). Statistical tests showed no significant difference in satisfaction with the treatment method in both groups (p > 0.05).

Discussion

The findings of the study showed that the mean of the reduction in the score of signs and symptoms of vaginal Candida infection was 7.4 ± 4.1 and 7.6 ± 3.8 in Artemisia and Clotrimazole group, respectively. The Wilcoxon test showed that improvement in the symptoms related to the vaginal Candida infection after treat-
ment in both groups using Artemisia and Clotrimazole was significantly higher than before treatment. There was no statistically significant difference between the two groups in terms of symptom improvement.

Also, the findings showed that the severity of symptoms of vaginal Candida infection before treatment was moderate in most of the studied patients in Artemisia and Clotrimazole group. The Mann-Whitney test did not show any significant differences. After treatment, all subjects in the Artemisia and Clotrimazole group had no symptoms of vaginal candidiasis. The Mann-Whitney test showed no significant difference between the two drugs. The Wilcoxon test revealed significant improvement in severity of vaginal Candida symptoms after treatment in both Artemisia and Clotrimazole group.

The results of statistical tests showed that there were no statistically significant differences in terms of family and personal characteristics, women's and midwifery information, information about candidacy, type of contraception and also health information between the two groups using Artemisia and Clotrimazole. They were homogeneous in this regard.

The mean score of symptoms of vaginal candidiasis was 7.4 ± 4.1 and 6.6 ± 3.8 in the Artemisia and Clotrimazole groups, respectively. There was no statistically significant difference between the two groups. The Wilcoxon test showed a significant reduction in the score of symptoms of vaginal candidiasis after treatment in both Artemisia and Clotrimazole group.

Concerning the effect of Artemisia and Clotrimazole on the secretion of Candida vulvovaginitis, the results of this study showed that both drugs have a significant reduction in secretion. Comparison of the effect of the two drugs on the reduction of secretion was not significant, which was consistent with the results of the Fouladi, Kordi, Hazrati and Roozbahani (28, 35-37). One of the results which is not consistent with the present research was published by Mousavi et al. In this study, the level of improvement in the Clotrimazole group was higher than in the bee propolis group which could be explainable due to the dosage of the prescription drug and the difference in the active ingredients of this herbal medicine. Due to concerns about the possible side effects, there was no possibility of further dose increase (38). In the study of Janani and Roozbahani, the rate of improvement in secretion in the Myrtus and "garlic" group was reported more than in the Clotrimazole group (39, 40). In the study of Janani, Myrtus was used with the concentration of 5%. However, two 500 mg capsules containing 5 grams of Myrtus were consumed by patients in the study of Roozbahani. Therefore, one of the reasons for the contradiction between the results of these two studies and the present study is the difference in the dosage administered in the Janani study and dose and method of consumption in the study of Roozbahani.

Concerning the effect of Artemisia and Clotrimazole on itching due to Candida vulvovaginitis, the results of this study demonstrated that itching was the main complaint in two groups, which is consistent with the results of the Janani, Roozbahani and Fouladi studies (28, 39, 40). The level of itching in the two groups was significantly decreased after the intervention. The results of the present study are consistent with the results of the Fouladi research, Bahadoran, Hazrati and Kordi (28, 35-37). Among the non-consistent results with the present study, we can mention the research of Mousavi, Janani and Roozbahani (38, 40). The possible cause may be due to the difference in dosage and active ingredients of the drug prescribed by the researchers.

Concerning the effect of Artemisia and Clotrimazole on burning due to Candida vulvovaginitis, the results of this study showed that both drugs reduced burning. Comparison of the effect of two drugs between the two groups was not significant. The results of the present study were consistent with the study of Janani, Fouladi, Kordi, Hazrati and Roozbahani (28, 36, 37, 39, 40). However, their results are not consistent with the results of Mousavi (38).

Concerning the effect of Artemisia and Clotrimazole on vaginal irritation and painful intercourse due to Candida vulvovaginitis, the results of this study revealed that both drugs reduced the above symptoms. There was no significant difference between the two drugs. The present study was consistent with the studies of Roozbahani Fouladi, Bahadoran, Hazrati and Janani (28, 35, 36, 39, 40).

Based on the post-treatment follow-up form, the samples did not report any side effects or deterioration of the symptoms. In a study by Fouladi, no cases of side effects were reported in consumption of Zataria multiflora boiss (Avishan Shirazi) vaginal cream (28). However, in Mousavi's study, 30% of the bee propol group participants and 15% in the Clotrimazole group complained of burning during treatment. In Kordi et al. study, 6% and 8% of complications were reported in the Clotrimazole and garlic extract groups, respectively (38).

The following reasons are the positive points of the present study: conducting a triple-blind study, the use of gold standard method for the diagnosis of Candida infection and vaginal discharge in a special laboratory of parasitology and mycology under the supervision of the
associate professor of mycology. In addition, the drug is used indigenously in Iran and is abundantly available in the pastures in the country, which is not an expensive treatment. Therefore, further research in this field can be used widely.

Limitations

The limitations of this study could be the researcher’s trust in patients regarding the use of drugs and observation of health standards correctly, the possibility of not reporting the symptoms accurately and the impossibility of long-term follow-up of the patients.

CONCLUSION

The present study showed that Artemisia and Clotrimazole vaginal creams improved the clinical symptoms related to Candida vaginal infection, and the therapeutic effects of these two drugs were similar. Therefore, the use of this drug as an alternative treatment in vaginal candidiasis is suggested. It is also suggested that more investigation is needed to study the effect of different species and doses of Artemisia in vaginal candidiasis treatment. The treatment of recurrent Candida infection is also suggested to be studied as well.

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**Poređenje efekata vaginalne kreme artemizije i klotrimazola na vaginalnu kandidijazu**

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**SAŽETAK**

Vaginalna kandidijaza je druga po učestalosti među vaginalnim infekcijama i leči se azolom. Prekomerno korišćenje azola dovodi do rezistencije i neuspešnog lečenja. Studije koje su sprovedene u laboratorijskim uslovima potvrdile su antifungalne efekte biljke artemizija. Cilj ove studije bio je poređenje efekata vaginalne kreme artimizije i klotrimazola u lečenju vaginalne kandidijaze.


Rezultati studije pokazali su da su srednje vrednosti smanjenja znakova i simptoma vaginalne kandidijaze za grupe koje su lečene artemizija kremom i klotrimazolom iznosile 7,4 ± 4,1 i 7,6 ± 3,8. Wilkinson test je pokazao da je u obe grupe lečene kremom artemizije i klotrimazolom poboljšanje bilo značajno veće nego pre lečenja (p < 0.001). Nije bilo značajne razlike između grupa u pogledu poboljšanja simptoma (p < 0.05).

Ova studija je pokazala da su i krem artemizije i klotrimazol poboljšali simptome vaginalne kandidijaze. Pored toga, nije bilo statističke značajnosti u terapijskom efektu oba leka. Iz tog razloga artemizija krem se može koristiti kao alternativa u lečenju vaginalne kandidijaze.

**Ključne reči:** Candida vulvovaginitis, artemizija, klotrimazol