

The comparative study of anti-fungal effect of pharmaceutical products containing hydroalcoholic extract of *Echinophora platyloba* DC and fluconazole in women with chronic recurrent vaginitis caused by *Candida albicans*

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BACKGROUND: The second most common vaginal infection is candida vaginitis. The use of anti-fungal traditional medicine and herbal therapies has been increased in recent years. The anti-fungal effect of *E. platyloba* DC extract on candida has been shown in the in vitro studies. The purpose of this study was to investigate this effect in women with candida vaginitis by in vivo study. **METHODS:** A randomized double blind clinical trial was done on 60 married women with candida albicans vaginitis referred to Obstetrics and Gynecology clinic. Patients were randomly divided into two groups. The first group received Echino cream plus fluconazole and the second group received fluconazole alone. Patients were evaluated by the second questionnaire and laboratory studies on vaginal specimens two weeks and 6 months later for recurrence. **RESULTS:** 14 days after treatment the positive culture of vaginal discharge were observed in 13 cases of (43.3%) the group treated with fluconazole, and 6 cases (20%) treated with fluconazole and Echino cream ($p < 0.5$). The rate of recurrent candida vaginitis was 17 cases (56.7%) in the group treated with fluconazole, and 8 cases (26.7%) in the second group ($p < 0.5$). **CONCLUSIONS:** *E. platyloba* DC extract was effective in women with candida vaginitis. More multicentral studies are needed to evaluate the effects in more details.

KEYWORDS: *Echinophora Platyloba*, *Candida Albicans*, Vaginitis, Recurrence, Herbal Medicine, Traditional Medicine

BACKGROUND

Vaginitis is the most common gynecological problem in gynecology and obstetrics that cause women to seek treatment.^[1] *Candida* vaginitis is the second most common vaginal infection by which 75% of women are affected at least once in their life.^[2] 40-50% of patients referred to the clinic with recurrence and a population of about 5% are affected by repeated infections throughout their lives.^[3] In 2009 Ahmad and Khan reported that the incidence of candida vaginitis in symptomatic women was 77% and in asymptomatic women it was 17%.^[4] Nowakowska et al. , in the same year, reported its incidence to be 62% in Poland.^[5] A similar incidence rate of candida vaginitis was shown in researches done in Iran. Molazade in 2001 reported its incidence to be 35% in Jiroft, Torabi and Amini reported a 48% incidence in Zanjan and Safari et al. reported a 34% incidence in Yasuj.^[6-8] Chemical topical antifungal medication consist of two different groups of azol and polyene.^[8] Non-pharmacological methods and some natural materials including ca

prylic acid, boric acid, lactobacillusacidophilus, vinegar therapy, and yogurt therapy could also treat yeast .^[9-10] recently, it has been shown that herbal medicine can help as an individual medicine or alongside Western medicine in the treatment of fungal diseases; for example in the treatment of cutaneous tinea and genital candidiasis a plant extract called *Agastache* enhances the effects of chemical drugs.^[11]

Echinophora platyloba DC is a native plant of Iran and it's antifungal effects are shown in several studies. Previous studies have shown that this plant contains saponin, flavonoid and alkaloid compounds.^[12] The most important identified terpene compound is trans-beta-Ausymn 67.9% .^[13,14] In two experimental studies *E. platyloba* DC extract has had a good effect on some dermatophytes and candida albicans yeast.^[12,15] Extract concentration of 35 mg per ml had an inhibitory effect on *C. albicans* growth.^[12] In another study the minimal bactericidal concentration of hydroalcoholic extract of the plant was determined to about 1.5 mg per ml.^[16] All past

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studies have investigated the antifungal effects of *E. platyloba* DC in vitro, and now with regard to these results, the antifungal effect of *E. platyloba* DC can be examined in clinical settings and in patients suffering from candida vaginitis.

METHODS

This study was a double-blind, randomized clinical trial which was done on 60 women with chronic and recurrent candida vaginitis and referring to the Clinic of Obstetrics and Gynecology (during the period of 2009 to 2011 in Esfahan, Iran). This project is approved by the research chancellery of Isfahan University of Medical Sciences under the research project number of 389402.

Inclusion criteria:

- 1 - Women with recurrent vaginitis (three or more attacks per year)
- 2 - Diagnostic tests had confirmed *C. albicans* as a cause of vaginitis.
- 3 - The absence of pregnancy, lactation and heart, kidney and liver disease.

exclusion criteria:

- 1 - Pregnancy during treatment
- 2 - Non-compliance to treatment
- 3 - Complication of drug

The patients were randomly allocated into two groups by the table of random numbers.

Echinophora platyloba DC (collection, extracts preparation):

E. platyloba DC is a spiny plant with flowers on a single stem, so that the external radiation canopies typically have male flowers and female flowers are in the middle. Their fruit is cylindrical.

The *Echinophora* species have white and yellow flowers. It has 10 species and is native to the

Mediterranean region. *Echinophora platyloba* D.C is used in the province of Chahar Mahal and Bakhtiari as a spice and flavor.^[14,15,16] This type is known with local names such as khosharuz, khusharizeh, tigh turagh, koshandar.^[17] Only four species of this plant are native to Iran. These species include:

E. cinerea, *E. sibthorpiana*, *E. orientalis* and, *E. platyloba*.^[18]

This plant grows from early September to October and then begins its sleeping season. It seems that alti-

tude and temperature of the province is effective in changing of growing season.^[12]

In this study the stems, leaves, and flowers of this plant were used. The samples were collected during summer (August and October). After collecting and drying the plant, it was used for preparing the extract.

The method of extracting from Echinophora platyloba):

The plant is dried. Then it is made into a powder. Then tiny particles are separated by sieve number 50 and the bigger particles by sieve number 10 in order to have a good powder of this plant. Then this powder is damped with a small amount of Ethanol 70. Then we place it in percolator. Then we add Ethanol 70 until it covers the plant. After 48 hours, we collect the extract and dry it if needed.

Formula:

Cream contains *Echinophora platyloba* extract:

1 - Oily phase:

a- Stearic acid 12%, b- Cetil acid 3.3%, c- Liquid paraffin 4%

d- BHT(Butylated hydroxytoluene) 2%, e- p.p (Propylparaben) 2%

2 - Watery phase:

a- Glycerin 8%, b- sodium lauryl sulfate 1.2%

c- Methylparaben 0.18%, d- water 67.08%

3 - The effective material:

Liquid extract of *Echinophora platyloba* 4.2%

Producing procedure:

The cream was formulated in development and research unit of Baryj Essence Pharmaceutical Company.

In a separate container we combined the oily phase and heated it to 72 degrees centigrade in order to melt all the substances and produce a monotonous solution.

In another container we combined the watery phase and heated it to 76 degrees centigrade in order to have a monotonous phase.

We add the watery phase to the oily phase and carefully mixed the solution.

We added the *Echinophora platyloba* extract to the solution when the temperature of the mixture was about 60 degrees centigrade.

Sampling procedure:

From patients with recurrent candida vaginitis referring to the Clinic of Gynecology and Obstetrics, based on

questionnaire information, entry criteria and diagnosis by a doctor, a sample of vaginal secretion was collected in a sterile cotton swab and each sample in a tube containing saline solution was transferred to the laboratory for diagnosis. After preparing the slides and gram stain, and finding budding yeast cells and *C. albicans* Pseudohyphae, for confirming the diagnosis, SCC-specific medium was used. In vitro, with growth of 1000 cfu / ml candida albicans, infection was considered.

After sampling and confirmation of recurrent vaginitis caused by *C. albicans*, patients were randomly divided into two groups of 30, In a way that they took number and Label based on the order of their arrival. For the first group Echino cream plus fluconazole was prescribed and for the second group fluconazole alone. The method of taking the drug is to use Echino cream at night before bed and one pill of fluconazole 150 mg at night. After two weeks of treatment, the response was reviewed in patients based on the second questionnaire and the laboratory studies on vaginal specimens collected, and the patients were examined 6 months later for recurrence. (Figure 1 and 2).

Software SPSS version 19 was used for data analysis.

RESULTS

All subjects were from suburban areas. Investigation showed that the largest age group was in the fourth decade of their life. 6 people (10%) were in the age group of 20 to 24.9, 4 (6.7%) patients in the age group 25-29.9, 17 (28.3%) in the age group 30-34.9, 22 (36%) were in the age group 35-44.9 and 11 (18.3%) were in the age group of over 45. 2 patients had no pregnancies, 6 cases (10%) had one, 12 cases (20%) had two, 16 cases (26.7%) had 3 and 24 patients (40%) had 4 pregnancies and more.

The cross-sectional tables of pregnancy and age indicated that the majority of women had four pregnancies and more and they were 35 years of age and higher. Most deliveries were normal vaginal and cesarean had been performed in only 11 people. All subjects had a history of previous vaginitis; most of them were infected more than 3 times.

In the group treated with fluconazole, 14 days after treatment culture of vaginal discharge was positive in 13 patients (43.3%), While in the group treated with fluconazole and Echino cream only 6 cases (20%) had positive culture. This difference was statistically significant ($p < 0.5$). Evaluation of the prevalence rate of re-

current candida vaginitis showed that in the group treated with fluconazole, 17 patients (56.7%) had recurrence. While the frequency of recurrence in the second group was 8 cases (26.7%) ($p < 0.5$).

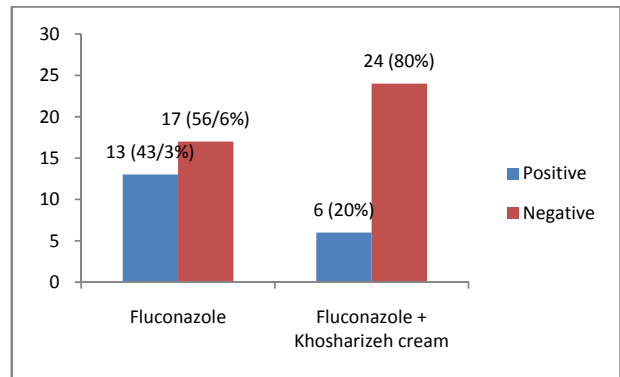


Figure 1. Frequency of positive culture results after 14 days of treatment in women with candida vaginitis

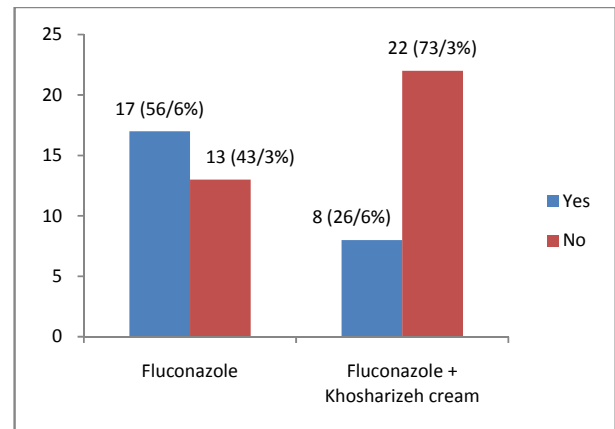


Figure 2. Frequency of recurrent candida vaginitis in women with candida vaginitis

DISCUSSION

Herbal medicine is a branch of traditional medicine. In countries like Iran it has about a hundred year history, and in the past it played a major role in treating diseases. With the advent of synthetic chemical drugs, herbal medications were gradually replaced. Fortunately, in recent years, the use of herbal medicine and drugs of plant origin has increased. Therefore, today about 1.3 to half of the pharmaceutical products in the markets of America have a natural origin.^[19,20] Moreover, the use of herbal medicine has also increased in England.^[21,22]

Not only people, but also clinical microbiologists are willing to use these drugs to treat infections, and this is due to the fact that the side effects of these drugs are significantly lower than chemical drugs.^[23,24] One of the medicinal plants that its anti-fungal effect has been shown in laboratory and clinical

cal settings and also for treating these infections is *Echinophora platyloba* DC.

In a study conducted by Avijgan et al. on the effect of various concentrations (5, 15, 25 and 35 mg per ml) of hydroalcoholic extract of *E. platyloba* DC on some skin fungi such as *trichophyton schoenleinii* and *trichophyton verrucosum* was evaluated. The results indicated that these two species were sensitive to all concentrations. *Trichophyton mentagrophytes*, *Microsporum canis* and *Epidermophyton floccosum* were sensitive only to the concentration of 25mg per ml. These results indicate that this plant's extract can have an antifungal effect.^[14]

In another in vitro study conducted by Mahbobi et al., the efficacy of *E. platyloba* DC extract alone and in comparison to amphotericin B, and also the synergism effect of these two drugs were tested on the yeast of *C. albicans*. Moreover, minimum growth inhibitory concentration (MIC) and minimum Fungicidal concentration of growth (MLC) were determined by using micro broth dilution.

MIC and MLC values of amphotericin B on *C. albicans* were 8 microg/ml and 2 microg/ml, respectively, while for hydroalcoholic extract of *E. platyloba* DC, these values, were 3125 microg/ml and 1569 respectively. With the use of a combination of drugs, the MLC and MIC for amphotericin B decreased to 1 and 2 microg/ml respectively, which indicates the synergistic effect of hydroalcoholic extract of *E. platyloba* DC with amphotericin B.^[25]

The pharmaceutical effect of *Echinophora platyloba* extract on candida vaginitis has not been shown in any studies yet. Nevertheless in 2 separate studies, the pharmaceutical effect of herbal vaginal cream of *Zataria multiflora* Boiss and other products taken from *Teucrium polium* smoke in the treatment of this infection was evaluated. Bonyadpour et al. in an in vitro study done by diffusion disk method evaluated the effect of 3 drugs consisting of fluconazole, clotrimazole and a product taken from *Teucrium polium* smoke on *C. albicans*. Six cases demonstrated resistance to clotrimazole and 47 cases showed resistance to Fluconazole. While all samples were sensitive to the product taken from *Teucrium polium* smoke.^[26] Fouladi et al. in a clinical trial attempted to compare the effect of herbal cream from *zataria multiflora* Boiss with clotrimazole cream in candida vaginitis, evaluated 73 patients, and treatment was done in both groups for 1 week. Then the response was evaluated. Regarding clinical symptoms in the *zataria multiflora* Boiss group compared

with the clotrimazole group, a significant difference was observed. In the first group, the complete recovery was 54.3% but in the clotrimazole group it was 47.4%.^[27]

In our study which is the first clinical trial, the clinical effectiveness of *E. platyloba* extract in the treatment of candida vaginitis was compared with fluconazole.

In the group treated with fluconazole 14 days after treatment the culture of vaginal discharge was positive in 13 patients (43.3%), while in the group treated with fluconazole and Echino cream only 6 cases (20%) had positive culture, which is half of the first group's treatment failure.

In another study, a high frequency of resistance to fluconazole has also been achieved. Pakshir et al. reported this resistance to be 44.8% in vaginal candidiasis in Shiraz.^[28]

In studies on antifungal therapy with fluconazole the following issues were raised:

First: these pathogenic species are increasingly resistant to fluconazole so that a significant percentage of this type of vaginitis treatment with fluconazole alone results in failure.

Second: recurrence of candida vaginitis treated with fluconazole is high. As in our study approximately 56% of those treated had recurrence.

These two problems, namely the treatment failure and relapse in a significant percentage of our patients treated with fluconazole and Echino cream were removed. Therefore, recurrence was decreased to about half of the previous situation and failure was decreased to more than half. These results show the synergistic effect of this extract with fluconazole.

Because our study is the first clinical trial and because only fluconazole was evaluated in it, more studies of clinical trial type and in multi central should be done, and a regional larger sample of women with this type of infection should be evaluated, and the effect of *E. platyloba* compared with other azoles such as itraconazole and clotrimazole in order to generalize the results.

CONCLUSIONS

Regarding the synergistic effects of this product with fluconazole, it is suggested that to produce vaginal

product from this extract, and to use this product in combination with azole in women suffering from chronic recurrent *C. albicans* vaginitis, could prevent the major problem of treatment failure and high recurrence rate in the follow up period.

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