

Therapeutic Effects of Bunium Persicum Boiss (Black Zira) on Candida albicans Vaginitis

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ABSTRACT

It has been a significant increase in the incidence of vulvovaginitis candidiasis in recent years. The rising prevalence of *Candida albicans* antimyotic resistance increased the need for new and safe drugs for the treatment of vulvovaginitis candidiasis. In this randomized, triple blind clinical trial of 90 non-pregnant women infected with Candidal vulvovaginitis were placed in two groups of Clotrimazole Vaginal Cream and Black Zira (N=45 and Clotrimazole Vaginal Cream and placebo (N = 45). Both groups were treated for 7 days. At the beginning of study, Clinical and laboratory signs and symptoms were registered 4 and 7 days after treatment by questionnaire, observation form and secretions medium culture results. Data were analyzed by chi-square, McNamar, Mann–Whitney and t- tests through SPSS version 21. There was a statistically significant difference in vaginal itching, irritation, soreness after the treatment between the two groups and much more in the study group (P < 0.001). However, the culture and wet mount (with KOH) results showed much improvement in the study group after treatment. The use of Zira Capsule with Clotrimazole vaginal cream was found more effective than Clotrimazole vaginal cream alone, in most common symptoms and signs of this vaginitis. Therefore, Zira capsule is suggested for the treatment of *Candida albicans* Vaginitis.

Key words: Vulvovaginal candidiasis, Black Zira, Bunium Persicum, Clotrimazole.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is a major public health problem affecting a large number of otherwise healthy women.(1) about three quarters of women during their reproductive age have at least one episode of Vulvovaginal candidiasis (VVC) and approximately half have two or more episodes(2). *Candida albicans* has been reported as the cause of VVC in 85-95% of cases (3) but other species such as *C. glabrata*, *C.krusei* and *C. tropicalis* are also encountered (4) whilst *Candida*

glabrata represents the most common cause of non-*albicans candida* vaginitis(3). This disease, not only affects physical and psychological health of patients, but also imposes significant financial expenditure and difficulties for marital relationships and may even lead to infertility and preterm birth (5, 6). Vulvovaginal candidiasis is not a reportable disease and, due to the high degree of self-treatment and available over-the-counter agents, it is not possible to elevate the exact incidence of this infection (2). The incidence of VVC in Iran is reported between 10% and 47 % (7-9).

Several antifungal drugs are available for treatment of vulvovaginal candidiasis which include nystatina and azol products such as miconazole, clotrimazole and fluconazole (10). Azole topical drugs are the most common existing treatment for VVC. Its side effects include increase of hepatic enzymes, painful urination, and depression, which may occur by drug systematic absorption. Side effects such as irritation or contact dermatitis may also be observed. It is prescribed with caution for patients suffering from hepatic or kidney disorders. Systemic administration of this drug during pregnancy has not been teratogenic for small animals under study, but it has been embryotoxic. Therefore, this drug should be used with caution during pregnancy and even breast-feeding, since it is not obvious whether it enters into mother's breast milk(11). However increasing prevalence of Vulvovaginal candidiasis resistance to antifungal and new predisposing diseases and the high incidence of RVVC and the difficulties controlling its occurrence with conventional anti-mycotic therapy constitute a strong medical need for the development of immunological treatments adding to, if not replacing, the current antifungal treatment (12, 13). Aromatic plants have been widely used in folk medicine. It is known that most of their properties are due to their volatile oils. Essential oils from many plants are known to possess antifungal activity (14). *Bunium persicum* (Black Zira) is a rich source of essential oils with different activities such as antimidant, antimicrobial and antifungal (15, 16).

METHOD AND MATERIALS

This study was a randomized, triple blind clinical trial in order to compare Black Zira (*Bunium persicum*) and placebo on signs and symptoms of vulvovaginal candidiasis. The study was conducted in clinics and health centre in Jiroft city in 2015. The sample size for comparison of two groups determined 90 women diagnosed with vulvovaginal candidiasis. The patients filled out a consent form to participate in the research, which was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, International Branch. The criteria to enter the study included:

- Being at age of 18 to 45.
- Having symptoms and signs of vulvovaginal candidiasis.

- Positive lab test results.
- Not being pregnant and breastfeeding.
- Not using OCP
- Not using broad-spectrum antibiotics in last two weeks
- Not using Immunosuppressant drugs
- Not using antimycotic drug in last four weeks

Exclusion criteria included

- Pregnancy
- Menstruation
- Abnormal vaginal bleeding
- Not using capsule for two doses or more.

Data collection tool in this study was a questionnaire on demographic variables and confounding background consists of three parts:

1. Demographic variables: (age, BMI, education, job, spouse's education, spouse's job, family income in month, housing)
2. Information about menstruation and pregnancy include (number of pregnancy, number of delivery, menstrual pattern, method of contraception)
3. Health information includes (Use sanitary pads, cloth napkins, tampons, pool, bath, public bath, a Jacuzzi, a stretch underwear, cotton or synthetic under wear, the use of tight underwear, drying after purification, the average number of times per week having intercourse)

In this research, at first History of patients was obtained and in case of having the signs of Vulvovaginal candidiasis (itching, secretion pain during intercourse and dysuria) preliminary questionnaire was completed. In case of no prohibition against the inclusion criteria, the study was fully explained to the patient. Then, with filling the written consent of the study the subject was placed in lithotomy position and sterile speculum placed into the vagina without being impregnated material and cervix and vagina were evaluated in terms of symptoms and cheesy white secretions erythema and even redness, sores and lesions , papulopustular damages and any abnormal findings. And it was recorded in the observations check list. Then with two sterile cotton swabs of vaginal swab, samples were taken at the top and side walls secretions.

The first swab was drawn on two slides, added a drop of normal saline on first slide and examined under the microscope (Nikon, USA) for the presence of clue cells and flagella trichomonas vaginalis (kapa 80%) and in case of observing the clue cells of Bacterial vaginosis or flagella trichomonas vaginalis, the sample was excluded from the study. And then on second slide was added 1 drop of KOH 10% solution and examined under the microscope. If Mycelium and blastopore observed, smear test was considered positive. Vaginal PH was measured by PH meter paper (Merck, Germany). Secretions PH above the 4.5 suggests mixed infection and were excluded from the study.

Samples were cultured on Chromagar medium (Paris, France) and incubated at 27-30 ° C for 24 to 48 hours and formed colonies on the medium after 72 hours. Then formed colonies were transferred to sabouraud dextroseagar medium and incubated at 27-30 ° C for 48 to 72 hours. The identification of the Candida species was done by sugar assimilation test with HiCandida identification kit (HiMedia, Mumbai, India). HiCandida identification kit was applied for precise identification of Candida species as per the manufacturer's instructions. The plastic strip had twelve wells with sterile medium for different biochemical tests as follow: well 1, medium for the urease detection test, and well 2 - 12, medium for carbohydrate utilization test (with eleven different sugars in respective wells, including, melibiose, lactose, maltose, sucrose, galactose, cellobiose, inositol, xylose, dulcitol, raffinose and trehalose). In brief, the test was performed as follows; at first a homogenous yeast suspension (1 to 5 × 10⁶ cell/mL) was prepared and incubated into kit wells and incubated for 24 - 28 hours at 22.5 ± 2.5°C. A standard sample of *C. albicans* (as confirmed by the molecular method) was also used as the control. After the incubation period the change of color in the kit was noted: well 1 containing urease was considered positive if the yellow color transformed to pink. Wells 2-12 were considered positive if their orange red color changed to yellow; these pits were left for 72 hours and if the color was still orange, the result was considered negative. Interpretation of the results was based on the manufacturer's instructions.

Positive culture result makes the diagnosis sure and all Candida species were entered to the study. The patients for treatment of vaginal candidiasis were randomly placed in two groups of 45, Clotrimazole vaginal cream and Black Zira group and Clotrimazole vaginal cream and placebo group. All patients were given the guidelines of drug use and health advice. They were told to apply creams each night as an applicator (5 g) inside the vagina for 7 nights. The patients were asked to refer to clinic 4 days after beginning of treatment and 7 days after completion of treatment.

The samples were evaluated microscopically. For final confirmation of the presence or absence of fungal, subculture of samples was prepared. Data was analysed by Chi-square, McNemar, Mann-Whitney and t-test through SPSS statistical software (21) and P < 0.05 considered as significant level.

Age, BMI and educational level were baseline variables. The populations mean age and BMI were 29 – 30 years and 22- 23 kg/mm. (Table 1) There was no significant difference (P = 0.98) between the two groups in educational level according to Chi-square test and most of them had diploma. Socioeconomic situation was an intervening variable measured by four variables including job (Chi-square, P = 0.66), housing- index (Chi-square, P = 0.64), income (Fisher exact test, P = 0.70) and number of family members (t-test, P = 0.48). There was no significant difference between the two groups.

According to Chi-square test, there was no significant difference (P > 0.05) between the two groups before treatment in symptoms including vaginal itching, discharge, irritation, dysuria and dyspareunia, soreness. But, according to Mann-Whitney test, there was a significant difference between two groups in terms of itching, discharge, irritation and soreness 4 days after beginning of treatment. (Table.2) In 4 days after beginning of treatment evaluation, patients were not able to respond about vaginal discharge due to applying vaginal cream, and they complained of discharge. Dyspareunia was not evaluated because the patients were asked not to have intercourse during

treatment period. Therefore, statistical analysis was not done for these two variables.

According to Chi-square test, there was no significant difference ($P \geq 0.05$) between the two groups before and after the treatment in symptoms including vaginal itching, discharge, dysuria and dyspareunia. However, all of these symptoms were different between the two groups after treatment ($P \leq 0.001$) and decreased more in

Zira group. According to McNemar test, significant differences were observed in the both groups before and after the treatment ($P < 0.05$) and based on Chi-Square test, significant differences were observed between the groups in soreness and vulva irritation ($P \leq 0.001$). (Table.3) According to table 2, Zira group symptoms were improved in 4 days after beginning of treatment which indicates study group improved in less time. ($P \leq 0.001$)

Table 1: Comparison of Mean Individual Characteristics in the Study and Control Groups

Groups	Black Zira Group n=45 Mean± SD	Placebo Group n=45 Mean± SD	Independent t-test P-Value
Age	30/81±6/76	29/23±7/31	0.311
Weight	63/13±11/57	61/60±9/86	0.501
Height	162/84±9/80	163/33±6/85	0.785
BMI	23/70±3/64	22/99±3/24	0.337

Table 2: Distribution of absolute and relative frequency of research units based on the symptoms, treatment phases and groups 4 days after beginning of treatment

	Before treatment			4 days after beginning of treatment		
	Zira	Placebo	p-value	Zira	Placebo.	P-value
Discharge	39 (86.7)	41 (91.1)	0.570	-	-	-
Itching	35(77.8)	37(82.2)	0.598	8(18.8)	23(51.1)	0.01
Irritation	39(86.7)	38(84.4)	0.764	16(35.6)	35(77.8)	0.00
Dysuria	17(8.9)	18(40.0)	0.829	4(8.9)	17(37.8)	0.01
Dyspareunia	35(77.8)	37(82.2)	0.598	-	-	-
Soreness	30(66.7)	29(64.4)	0.824	9(64.4)	20(46.4)	0.02

Table 3: Distribution of absolute and relative frequency of research units based on the symptoms, treatment phases and groups 7 days after the intervention.

	Before treatment			7 days after treatment		
	Zira	Placebo	p-value	Zira	Placebo.	P-value
Discharge	39 (86.7)	41 (91.1)	0.570	7(15.6)	10(22.2)	0.82
Itching	35(77.8)	37(82.2)	0.598	2 (4.4)	8(17.8)	0.28
Irritation	39(86.7)	38(84.4)	0.764	4(8.9)	31(68.9)	0.00
Dysuria	17(8.9)	18(40.0)	0.829	2(4.4)	2(4.4)	0.48
Dyspareunia	35(77.8)	37(82.2)	0.598	2(4.4)	5(11.1)	0.97
Soreness	30(66.7)	29(64.4)	0.824	3(6.7)	18(4.0)	0.00

Table 4: Comparison of Distribution of lab Tests in the Study and Control Groups

	Before Treatment			After Treatment		
	Zira	Placebo	P	Zira	Placebo	P
Wet mount (KOH)	45(100)	45(100)	<0.001	11(24.4)	26(57.7)	0.00
Chromagar Culture	45(100)	45(100)	<0.001	9(20.5)	23(51.1)	0.00

There was not a significant difference ($P \leq 0.05$) between the two groups for Wet mount with KOH and culture test with Chromagar before the treatment. Wet mount of patients with normal saline had always negative results before and after the treatment, so there was no statistical analysis. According to McNemar test and Chi-Square, significant differences were observed between the both groups before and after the treatment for wet mount with KOH and Chromagar culture ($P < 0.001$).

DISCUSSION

It has been a significant increase in the incidence of vulvovaginitis candidiasis in recent years. The rising prevalence of candida albicans antimyotic resistance increased the need for new and safe drugs for the treatment of vulvovaginitis candidiasis. However there are some in vitro and in vivo researches on Black Zira and its medicinal effects, there is no study about the effect of this herb on candida albicans vaginitis in human. The present study is conducted to compare the effects of Black Zira (*Bunium persicum*) and placebo on signs and symptoms of vulvovaginal candidiasis in 4 days after beginning of treatment and 7 days after completion of treatment. In the present study, the most common complaints in both groups was vaginal discharge which is in agreement with Bahadoran study(17). But researchers in Michigan University stated itching as the main cause of referring patients to clinics(18). Jonthan S. Berek stated vulva itching and vaginal discharge as the most common symptoms of vulvovaginitis candidiasis(19).

The obtained results indicate that all signs and symptoms of vaginal candidiasis reduced significantly 4 days after beginning of treatment and

7 days after completion of treatment. But the differences in soreness and vulva irritation were more significant than the other symptoms. According to Yahya Zadeh and etal, Methanol extracts of Black Zira has analgesic effect on rats because of presence of Flavonoids in Methanol extracts of Black Zira. Haji Hashemi (2011) study indicates that Black Zira has strong analgesic and anti-inflammatory effects and impact on dysmenorrhea which is in agreement with this study (20). Wet mount of patients with normal saline had always negative results before and after the treatment and PH of vagina was natural before and after the treatment in both groups.

Significant differences were observed in the both groups before and after the treatment for wet mount with KOH and Chromagar culture ($P < 0.001$). It means that Clotrimazole cream and Black Zira affects more on negative fungal growth after treatment than Clotrimazole vaginal cream and placebo group. According to Sekin (2007) and et al study, among 52 different herbs, Black Zira has strongest anti-fungal effect due to anti-fungal impact of cuminaldehyde in Methanol extracts of Black Zira (15). Also in Naseri study in (2014), it is mentioned that Black Zira inhibitory power is significantly more than fluconazole in vitro(21). In an investigation conducted by Gholami et al. (2012) it was shown that black Zira can inhibit growth of *C.glabrata* isolates which were resistant to fluconazole(22).

CONCLUSION

This study implies scientific evidence of using Black Zira in folk medicine. It also can be a base for further studies for developing effective anti-fungal drugs. Finally, regarding the findings of this study, this drug can be used with confidence for

treatment of candidiasis vaginitis in patients who tend to treatment with herbal medications or cases resistance to Clotrimazole.

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