ORIGINAL ARTICLE

The effect of *Calendula officinalis* versus metronidazole on bacterial vaginosis in women: A double-blind randomized controlled trial

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J. Adv. Pharm. Technol. Res.

ABSTRACT

Bacterial vaginosis (BV) is a common disorder among women of reproductive age. This study aimed to compare the effect of a *Calendula officinalis* extract-based cream and metronidazole on BV among women of reproductive age. In this study, 80 women of reproductive age with BV were randomly assigned to the *C. officinalis* (n = 40) or metronidazole (n = 40). Diagnosis of BV was confirmed when at least 3 of the 4 Amsel criteria were met (pH >4.5, whitish grey or thin homogeneous discharge, release of a fishy odor on adding 10% KOH, and detection of clue cells on microscopic examination). For each group, either a methanol extract of *C. officinalis* or metronidazole vaginal cream (5 g) was used for 1 week intravaginally, and all signs and symptoms were assessed 1 week after treatment completion. Before the intervention, the two groups did not differ significantly with regard to vaginal burning, odor, dysuria, and dyspareunia, but itching was significantly more common in the *C. officinalis* group than in the metronidazole group (22.5% vs. 2.5%, P = 0.01). One week after the intervention, all women in both groups were free of symptoms, including vaginal itching and burning sensation, odor, dysuria, and dyspareunia. None of the women in either group suffered any side effects from *C. officinalis* or metronidazole. *C. officinalis* was effective for the treatment of BV in women of reproductive age, without any side effects. This herb could be recommended for women of reproductive age who uncomfortable with the potential side effects of synthetic drugs.

Key words: Bacterial, *Calendula officinalis*, metronidazole, vaginitis

INTRODUCTION

Bacterial vaginosis (BV) is a common disorder among reproductive-aged women, and its underlying cause is primarily a change in vaginal flora.[1] BV is characterized by the suppression of the normal vaginal flora (hydrogen peroxide-producing *Lactobacillus*) and an increase in facultative and anaerobic bacteria.[2] The main cause of BV is unclear; however, it is more commonly seen with anaerobic bacteria than with aerobic bacteria.[3]

The prevalence of BV was reported to be 29.2% in a large study in the United States, which corresponds to 21.4 million women.[4] One study in Iran showed that the prevalence of BV among reproductive-aged women was 16.2%[5] while another study in Iran found a prevalence of 15.6% among these women.[6]

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Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/japtr.JAPTR_305_17

How to cite this article: Pazhohideh Z, Mohammadi S, Bahrami N, Mojab F, Abedi P, Maraghi E. The effect of *Calendula officinalis* versus metronidazole on bacterial vaginosis in women: A double-blind randomized controlled trial. J Adv Pharm Technol Res 2017;9:15-9.

Because of the unpleasant odor associated with BV, most women experience embarrassment and feel dirty and believe that the smell can be detected by others. In addition, women with BV have low self-esteem and lower sexual satisfaction.[7] BV can be treated with a variety of antimicrobial agents, such as ampicillin, penicillin, and metronidazole. However, metronidazole in the form of tablets, gel, or suppositories used for 7 days shows better results.[8] The use of antimicrobial agents has been associated with drug resistance and in some patients, recurrence of BV.[9] Furthermore, using metronidazole during pregnancy and while breastfeeding may pose some risks to the fetus and infant.[10] Therefore, researchers are looking for alternative treatment for BV. One alternative is the use of herbal medicine, which is associated with fewer complications and which could preserve the natural flora of the body.[11] *Calendula officinalis L* (pot marigold) is a herbaceous plant from the Asteraceae family that contains phytochemicals such as flavonoids, saponins, carotenoids, triterpenoids, and tannins, which possess antibacterial activity.[12] Bissa and Bohra studied the antibacterial potential of the leaves, roots, and flowers of *C. officinalis* and found good activity against *Escherichia coli*, *Salmonella*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Agrobacterium tumefaciens*.[13] The anti-inflammatory, antifungal, and antioxidant effects of *C. officinalis* have been demonstrated, but there is a lack of studies regarding its effect on BV. Therefore, the aim of this study was to compare the effect of *C. officinalis* and metronidazole vaginal cream on BV among reproductive-aged women.

MATERIALS AND METHODS

This was a double-blind randomized controlled trial for which 80 nonpregnant reproductive-aged women in Dezful, Iran, were recruited. The study setting was a public health center in Dezful, Iran (Health center no: 3). The study protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ref no. IR.AJUMS.REC.1394.302). In addition, the protocol was registered in the Iranian Registry for Randomized Controlled Trials (Ref no. IRCT2015082921414N3). Written informed consent was obtained from all women before data collection. This study was started in early May 2016 and ended in late July 2016. The inclusion criteria were women aged 18–45 years who were married and had BV. Women who complained of other types of vaginitis or were pregnant were excluded from the study.

Sample size

The required sample size with regard to recovery after using metronidazole and *C. officinalis* (70 and 80%),[14] with a maximum acceptable error of 0.3 either side and a 20% attrition rate, was calculated to be 40 for each group.

Herbal extract

The fresh flowers of *C. officinalis* were purchased from an Iranian herbal market, and they were converted to a powder at the Pharmacy School of Shahid Beheshti University of Medical Sciences. The *C. officinalis* vaginal cream was made using the stages of extraction and formulation. The first and second stages of extraction were as follow: the minimum inhibitory concentration (MIC) was determined and the vaginal cream was prepared. All drug preparation processes were performed in the Bacteriology Department of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The mean MIC (0.07 μmol/ml) was considered against particular bacterial strains in this study. The direct method, in which the concentration of the extract was determined and the drug then added, was used to add the plant extract to the culture. Considering the antibacterial potency of the methanol extract (MIC = 0.079 μmol/ml) compared to that of the ethanol extract (MIC = 0.125 μmol/ml) and considering the lower cost of methanol, the methanol extract was chosen for the formulation. Composition of base cream was cetostearyl alcohol, petroleum jelly, glycerin, mineral oil, preservative, and antioxidant. After preparation, 80 tubes of cream were made in two stages with the necessary testing done in each stage.

Randomization

A total of 80 women with BV were randomized into the *C. officinalis* and metronidazole groups. Permuted block randomization with a block size of 4 (using a random permutation table) was used. The metronidazole and *C. officinalis* labels were encoded as A or B by a person who was not aware of the purpose of the study. The *C. officinalis* and metronidazole formulations were similar in appearance and consistency. The person who distributed metronidazole and *C. officinalis* was not aware of the purpose of study.

Intervention

A detailed history was taken from all patients who were seen at the health centers and a physical examination was performed. Vaginal pH was measured using pH strips. A Pap smear was then performed on all women who gave consent. Clinical signs and symptoms such as edema, inflammation, and changes in vaginal discharge were recorded for each participant. Next, a sample of vaginal discharge was obtained to look for clue cells and perform a whiff test using 10% KOH. Diagnosis of BV was confirmed when at least 3 of the 4 Amsel criteria were met (pH >4.5, whitish gray or thin homogenous discharge, release of a fishy odor on adding 10% KOH, and presence of clue cells in microscopy). Women who were diagnosed with BV were randomly treated with metronidazole or *C. officinalis* coded as A or B. All women in both groups received advice regarding avoiding intercourse, using cotton underwear, changing underwear regularly, keeping the perineum dry, and instruction on washing the applicator before starting treatment and after each use. Each woman in both groups

received a tube of cream containing 50 g *C. officinalis* or placebo, and women were instructed to use one applicator of vaginal cream (5 g) before sleep for 1 week. The effect of treatment was assessed 1 week after completion of the treatment through an interview, observation, and laboratory testing of vaginal discharge. If a patient did not attend the follow-up, one of the investigators called her, and if she still did not attend, she was considered a dropout. The vaginal discharge samples were assessed using a microscope and appropriate tests. All data were entered in the medical records of the patients. All women were asked to report any side effects of the treatment.

Statistical analysis

All data were entered in Chicago, Illinois: SPSS Inc., USA. The normal distribution of data was evaluated using the Shapiro–Wilk test. The independent *t*-test or Mann–Whitney test was used for comparing continuous data between the two groups while the Chi-square test was used for categorical data. $P < 0.05$ was considered statistically significant.

RESULTS

None of the participants withdrew from the study [Figure 1]. Table 1 shows the sociodemographic characteristics of the participants of the two groups. The median age of the women in the metronidazole and *C. officinalis* groups was 31 and 32.38 years, respectively. Almost all women (99.9%) in both groups used one method of contraception. The method that most women used was withdrawal (used by 35% of women in each group). Women did not show any significant difference in age, marriage age, body mass index, parity, amount of coitus per month, job, history of BV, and method of contraception.

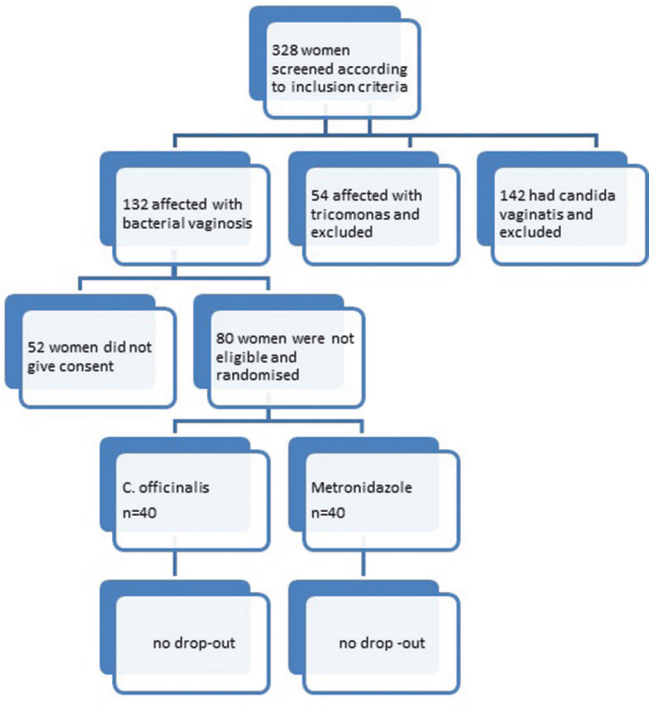

Figure 1: Flowchart of recruitment and retention and participants in the study

Before the intervention, most women in both groups had symptoms such as vaginal burning, itching, and discharge; odor; and dysuria, with dysuria being the most common in both groups. Itching was significantly more common in the *C. officinalis* group than in the metronidazole group (22.5% vs. 2.5%, $P = 0.01$). The two groups did not show any significant difference with regard to other symptoms before intervention. One week after intervention, all women in both groups were free of symptoms [Table 2]. None of the women in either group suffered any side effects from *C. officinalis* or metronidazole.

DISCUSSION

This study aimed to compare the effect of *C. officinalis* and metronidazole on BV in reproductive-aged women. Our results showed that both *C. officinalis* and metronidazole could eliminate all symptoms of BV in women. In support of our findings, Roopashree *et al*. showed that an aqueous extract of *C. officinalis* had an antibacterial effect against both Gram-positive and Gram-negative strains.[15] In addition, *C. officinalis* has shown that antifungal activity was as effective as clotrimazole in women with a *Candida albicans* infection.[16] In the present study, we used fresh flowers of *C. officinalis*. Other studies have shown that all types of extracts (aqueous, alcoholic, chloroform, and petroleum ether) of the leaves and flowers of *C. officinalis* were more active than extracts of the roots of this plant.[13]

In a study, Tedeschi *et al*. found that vaginal gel comprising isoflavones, *Lactobacillus sporogenes*, and *C. officinalis* could significantly reduce the signs and symptoms of vaginal dystrophy (itching, burning, vulvovaginal erythema, and vaginal dryness) compared to placebo in postmenopausal women.[17] Palmieri *et al*. found that a mixture containing Saliva, Melaleuca, and Calendula plants had a very good effect on the vaginal surface of women with damage to their vaginal mucosa. Furthermore, they found that Calendula could enhance the body's defense against external agents and exert anti-inflammatory, antioxidant, and wound healing effects.[18] The results of the aforementioned studies are in line with ours. In the present study, we used a methanol extract of *C. officinalis*, and other studies have shown that the methanol extract of *C. officinalis* had better antimicrobial activity than that of the ethanol extract.[19] Our study showed that women in both groups did not suffer any side effects from metronidazole or *C. officinalis*. Other studies have also demonstrated that the methanol extract of *C. officinalis* has very few side effects on the skin, such as skin irritation, with no sensitization or phototoxicity.[20]

Table 1: Demographic characteristics of participants in two groups of metronidazole and *Calendula officinalis*

Variables	Median (IQR); Range or *n* (%)		*P*
	Calendula officinalis (*n*=40)	Metronidazole (*n*=40)	
Age (year)	32.38 (26.2-37.5); 29	31 (28-36); 22	0.63
Marriage duration (year)	9.5 (5.25-15); 32	11 (5.2-17.7); 26	0.96
Body mass index (kg/m^2)	27.6 (24.3-31.1); 26	26.7 (24.1-29.4); 17	0.29
Gravida	2.03 (1-2.75); 8	2 (1-3); 6	0.28
Para	1 (1-2); 8	2 (1-3); 5	0.09
Number of children	1 (1-2); 5	2 (1-3); 5	0.06
Number of coitus per month	10 (8-13.5); 20	10 (7.25-14); 20	0.92
Job			
Housewife	37 (92.5)	39 (97.5)	1
Employee	3 (7.5)	1 (2.5)	
Mode of delivery			
Normal vaginal delivery	15 (37.5)	26 (65)	0.71
Cesarean	25 (62.5)	14 (37.8)	
History of bacterial vaginosis			
Yes	22 (55)	29 (72.5)	0.16
No	18 (45)	11 (27.5)	
Method of contraception			
Oral contraception pills	2 (5.7)	4 (10.8)	0.73
Depot medroxyprogesterone acetate	3 (8.6)	0	
Condom	6 (17.1)	7 (18.9)	
Tubal ligation	3 (8.6)	3 (8.1)	
Withdrawal	14 (37.8)	14 (40)	
Vasectomy	1 (2.9)	1 (2.7)	
Intrauterine device	6 (17.1)	8 (21.6)	
No contraception	5 (12.5)	3 (7.5)	

The *P* values are based on the results of Mann-Whitney U-test or Pearson's Chi-square test. IQR: Interquartile range (25th-75th percentiles)

Table 2: Clinical signs and symptoms before and after treatment in two groups of *Calendula officinalis* and metronidazole

Variables	*Calendula officinalis* (*n*=40)		Metronidazole (*n*=40)		*P* value before intervention
	Before, *n* (%)	After, *n* (%)	Before, *n* (%)	After, *n* (%)	
Burning					
Yes	13 (32.5)	0	18 (45)	0	0.35
No	27 (67.5)	40 (100)	22 (55)	40 (100)	
Itching					
Yes	9 (22.5)	0	1 (2.5)	0	0.01
No	31 (77.5)	40 (100)	39 (97.5)	40 (100)	
Vaginal discharge					
Yes	11 (27.5)	0	24 (60)	0	0.06
No	29 (72.5)	40 (100)	16 (40)	40 (100)	
Bad odor					
Yes	21 (52.5)	0	16 (40)	0	0.37
No	19 (47.5)	40 (100)	24 (60)	40 (100)	
Dysuria					
Yes	39 (97.5)	0	40 (100)	0	1
No	1 (2.5)	40 (100)	0	40 (100)	
Dyspareunia					
Yes	0	0	3 (7.5)	0	0.24
No	40 (100)		27 (92.5)		
pH*	4.7±0.25 (4.5-5.5)	4.7±0.23 (3.5-4.5)	4.9±0.28 (4.5-5.5)	4.1±0.28 (3.5-4.5)	0.58

*The values are expressed as mean±SD (range). The *P* values are based on the results of Fisher's exact test or independent *t*-test. SD: Standard deviation

Strengths and limitations of study

Although other studies have shown that *C. officinalis* is effective against vaginal candidiasis,[15] to the best of our knowledge, this is the first time that *C. officinalis* was compared with metronidazole for the treatment of BV. We relied on participant responses regarding how the vaginal cream was used and the presence of symptoms. These answers might have been affected by recall bias.

CONCLUSION

C. officinalis is effective for the treatment of BV in reproductive-aged women without any side effects. The use of this herb could be recommended in women who are uncomfortable with the potential side effects of conventional pharmaceuticals.

Acknowledgment

We would like to thank Ahvaz Jundishapur University of Medical Sciences for financial support of this study. Thanks also extended to women who participated in this study.

Financial support and sponsorship

This study financially supported by Ahvaz Jundishapur University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

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