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## Effect of *Hypericum perforatum* L. compared with metronidazole in bacterial vaginosis: a double-blind randomized trial

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## PEER REVIEW

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**Comments**

This work represents an important study. These researchers have tested *H. perforatum* vaginal gel on 162 married women 18–49 years old. They found that this gel exhibits a very interesting activity against the bacteria responsible for bacterial vaginosis. In all the cases, the originality of this work gave us a great satisfaction on the importance of this study in a number of biological and medicine fields.

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## ABSTRACT

**Objective:** To compare effect of *Hypericum perforatum* (*H. perforatum*) vaginal gel with metronidazole on bacterial vaginosis (BV) in terms of initial response to treatment and preventing recurrence (primary outcomes) and also patient complaints (secondary outcomes).

**Methods:** In this double-blind, double dummy trial, married women aged 18–49 with BV were randomized into two groups and administered 5 g of 3% *H. perforatum* and placebo of metronidazole ( $n=82$ ), or 5 g of 0.75% metronidazole and placebo of *H. perforatum* ( $n=80$ ) vaginally for 5 d. Amsel criteria were used for diagnosis and assessing cure and recurrence of BV. The comparisons was done using *Chi*-square, Fisher's exact and logistic regression.

**Results:** At 10–12 d, cure rate was 82% in the *H. perforatum* and 85% in metronidazole group (risk ratio 0.9, 95% confidence interval 0.6 to 1.3). Among the cured women, recurrence rate was 9% in the *H. perforatum* and 13% in the metronidazole group at the 30–35 d visit (risk ratio 0.8, 95% confidence interval 0.4 to 1.3). There was no statistically significant difference between the groups regarding any patient complaints, except itching which was less in *H. perforatum* group (5% vs. 16%,  $P=0.018$  at the first and 13% vs. 43%,  $P<0.001$  at the second follow-up). No significant adverse event was reported at any groups.

**Conclusions:** *H. perforatum* could be a good option for treatment of BV. However, further studies are needed for its public use.

## KEYWORDS

*Hypericum*, Metronidazole, Vaginosis, Bacterial, Therapy, Recurrence

### 1. Introduction

Bacterial vaginosis (BV), the most common cause of vaginal discharge among women in reproductive age[1], is indeed a change in the normal vaginal flora characterized

by a reduction or absence of the hydrogen-peroxide producing lactobacilli and an overgrowth of other bacteria, especially anaerobic species. It is associated with several complications, including sexually transmitted diseases and abnormal cervical cytology[2].

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Drug of choice for treatment of BV is metronidazole[2]. Its success rate is about 70 to 80 percent[3], but in 30% of patients with an initial response to treatment, the disease will recur within three months[4]. Also, its use is associated with some side effects such as drowsiness, dizziness, nausea, transient neutropenia, peripheral neuropathy and vaginal candidiasis[5]. In addition, allergy and resistant to the drug is seen in some patients[4,6].

In recent years, due to people's interest in herbal medicines and the high prevalence of side effects of chemical drugs, herbal medicine has had a growing trend[7]. Many studies have shown the efficacy and safety of some herbal medicines in different disorders including BV[8].

*Hypericum perforatum* L. (*H. perforatum*) which is commonly known as St John's wort is one of the plants that has attracted the attention of a lot of researchers nowadays, due to its numerous benefits. It is now one of the most widely used herbal medicines throughout the world[9]. It belongs to Hypericaceae family and is herbaceous and perennial plant. Its flowering branches are medicinally valuable[10]. This plant contains many compounds and chemical substances such as anthraquinone (naphthodianthrones) derivatives, flavonoids, pernilated phloroglucinols, tannins, some phenols, volatile oils, hyperforin and hypericin. Standardization of the plant and the industrial medicines produced from this plant are carried out based on two hypericin and pseudohypericin materials[11].

This plant is traditionally used for the treatment of inflammation of the bronchi and urogenital tract, biliary disorders, common cold, migraine headaches, sciatica, dyspepsia, malaria, and topically for cuts, burns and skin ulcers. Also, it is used for treatment of menstrual disorders, elimination of leukorrhea and bladder irritation and as an antidepressant, a diuretic and an analgesic[10]. In addition, different pharmaceutical dosage forms of this plant are prepared by some pharmaceutical companies like Golarou in Iran and Esensa d.o.o. company in Serbia[12,13], and are recommended for treatment of different disorders including microbial infection of skin and mucosa. In the study setting, this plant in different forms including brewed or incense forms is traditionally used to treat different types of infections including bacterial vaginosis, and most people are very satisfied with it.

No serious adverse event has been reported in human studies using *H. perforatum* (in form of oral drop, oral tablet, and topical ointment) on some other problems like scalp wound[14], premenstrual syndrome[15], pain of caesarean section[16]. Also, no side effect has been reported in its medicinal use[14–16], but there are probability of increasing sensitivity to light and some complications such as gastrointestinal irritations, allergic reactions, tiredness and sleep disorders in its use in larger amounts which are transient[10].

According to our bibliographic studies in the literature, there is no study on antibacterial effect of *H. perforatum* in

human. However, *in-vitro* studies showed that its extract has potent anti-bacterial effects[10,17–19].

The aim of this study was to assess effect of *H. perforatum* vaginal gel compared with that of metronidazole in BV in terms of initial response to treatment and preventing recurrence (primary outcomes) and also patient complaints (secondary outcomes).

## 2. Materials and methods

### 2.1. Participants and setting

This double-blind double-dummy randomized controlled trial was conducted on 162 married women aged 18–49 years old with bacterial vaginosis attending health centres in Jolfa and Hadishahr, two small cities located in East Azerbaijan province, Iran. The excluding criteria were pregnancy; lactation; smoking; suffering from sexually transmitted infections, candidiasis or heart or kidney diseases; expected menstruation within next 10 d; abnormal vaginal bleeding; having sexual intercourse, douching and vaginal drug use within the past 48 h; history of drug hypersensitivity to metronidazole; use of combined oral contraceptives, immune-suppressive, broad-spectrum antibiotics, anti-prostaglandins and anticoagulant drugs during the past month.

### 2.2. Recruitment and data collection

Final decision for eligibility and all clinical examinations of participants (including discharge sampling, whiff test and the pH measurement) were carried out by one investigator [Zahra Mohammadzadeh (ZM), one of co-authors], a midwife with long clinical experience working at the Jolfa social security clinic in the mornings and at a private office in the evenings. Midwives working at public health centres of the two cities referred potential eligible subjects to her working places after a brief explanation of research.

The investigator completed a questionnaire containing questions on demographic and reproductive characteristics and patient complaints by interview, after a more thorough description of objectives and study method, eligibility assessment and getting patient verbal informed consent. Then, she examined vagina and cervix regarding the signs of inflammation and discharge characteristics (colour, odour, consistency and rate of discharge) and the initial rule out of sexually transmitted infections and candidiasis.

In patients with a high risk of bacterial vaginosis who had no signs of other vaginal infections, discharge in the lateral vaginal walls and posterior fornix were taken by two sterile swabs. Discharge on the first swab was placed in a sterile tube containing 0.2 mL normal saline solution and was sent to the laboratory to examine the clue cells and trichomoniasis. Discharge on the second swab was placed on a clean glass

slide and after adding one drop of 10% potassium hydroxide (KOH) solution the slide was immediately investigated regarding the fishy odour (whiff test). Then it was covered with a cover slip and viewed directly under microscope for candidiasis. Moreover, pH of vaginal discharge was measured by a pH-meter tape (Merck, Germany) having 0.5 precision.

All slides were investigated by one laboratory specialist with no knowledge of the results of clinical assessment. The laboratory results were reported to ZM on the day of sampling.

At the same night she called the diagnosed infected persons and invited them to come to the social security clinic the next morning or to her private office in the evening to receive the treatment.

The diagnosis criteria of BV was based on the presence of three out of four Amsel criteria, *i.e.* homogeneous, thin, greyish-white or milky vaginal discharge; vaginal pH greater than 4.5; positive whiff test and presence of clue cells on microscopy. This method has high and acceptable sensitivity and specificity in BV diagnosis<sup>[20]</sup>.

Content validity of the data about collection tool was determined by eight experts. Vaginal discharge pH of 10 subjects were measured independently by two people (ZM and a gynaecologist) to determine the reliability of the pH-meter. Moreover, clinical examination for the 10 subjects was performed by two people and two separate vaginal samples were prepared from 10 subjects and were sent to the laboratory with two different names. There was over 90% consensus in all cases.

### 2.3. Randomization and intervention

The participants were randomly allocated into two groups using permuted block method with block sizes of 4 and 6 and allocation ration of 1:1 after obtaining an informed written consent. Allocation sequence was identified using computerized random numbers by a person not involved in recruitment, data collection and analysis. All participants got two 40-gram tubes of vaginal gels to use for 5 d; one tube of 3% *H. perforatum* and one tube of placebo of metronidazole, or one tube of 0.75% metronidazole and one tube of placebo of *H. perforatum*. The gels had been placed in identical sequentially numbered packages which were given to the participants in their recruitment order in the study. Reason for the placebo use was that it was not possible to make the two drugs identical in order to maintain blinding. The placebos were identical with their main drugs in every aspect except the active ingredient.

The participants were instructed to use the gels vaginally twice a day; one full applicator (5 g) each time, once in the mornings from one tube and once at nights from the other tube. They were also told to abstain from sexual intercourse or use condoms for 10 to 12 d, avoid douching and use no antibiotic or other vaginal creams or tablets during treatment

period. The patients were asked to fill up a diary about drug use and any side event and to inform any serious side event immediately by phone or in person.

### 2.4. Follow-up visits

The participants were asked to attend 10 to 12 d after starting the treatment to be re-assessed and deliver drug empty tubes. In case of negative response to treatment (positive result of at least two out of four Amsel criteria at the first follow-up)<sup>[21]</sup>, patients received the routine treatment with metronidazole tablets 500 mg, twice a day for 7 d. In case of treatment success, patients were notified by phone and asked to revisit 30 to 35 d after starting the treatment while abstaining from sexual intercourse for 48 h prior to the visit. At the follow-up visits, patient complaints were asked and clinical and laboratory assessments were done like the baseline stage.

### 2.5. Ethical considerations

Participant recruitment was started after approving research project scientifically by the Research Center of Infectious & Tropical Diseases (code: 9108) and ethically by ethics committee of Tabriz University Medical Sciences-Iran (code: 9060, date: Dec 25, 2011) and registering in Iranian registry controlled trial with IRCT201112063706N9 code (<http://apps.who.int/trialsearch/trial.aspx?trialid=IRCT201112063706N9>).

Written informed consent was got from all participants. In addition, prior to the study, the prepared gel was used by five members of the research group's close relatives with careful observance to make sure of lack of any serious side effects.

### 2.6. Drug preparation

In this study, we used 3% concentration of *H. perforatum* L. vaginal gel because it was used for the first time on human mucosa. In a study using *H. perforatum* L. concentrations of 2%, 5% and 20%, it was found that the effect of 2% concentration is better in rabbit's wound healing<sup>[22]</sup>.

The *H. perforatum* plant was purchased from a person with full knowledge of regional medicinal plants who had handpicked it from the Hadishahr region. The plant identification was confirmed by an expert working in medicinal plants research centre of the Tabriz University of Medical Sciences. In industrial pharmacy laboratory of pharmacy school of this university, the branches of the flowering plant (flowering tops) were soaked in 70% hydro-alcoholic solution and was stirred on a shaker for 72 h. After this period, the extract was filtered by paper filter and the remaining pulp was shaken again in the hydro-alcoholic solution for another 72 h and its extract was separated and

added to the previous extract. The obtained extract was placed in the rotary evaporator at 40 °C till all the solvent was removed and a dry powder was left. The obtained powder was converted into fine particles by milling.

For preparing the gel, sodium carboxymethylcellulose (4% w/w) was used. For this purpose, firstly the conserved water was prepared using the methyl and propyl-paraben. An appropriate amount of extracted powder was added to the water and was stirred until it was completely dissolved. Then sodium carboxymethylcellulose was added and stirred to obtain a gel with suitable viscosity. The phosphoric acid 1N was added drop by drop to the gel until the pH was adjusted to 4. The obtained gel was poured in sterile tubes which did not bear any names by filling machine.

The metronidazole vaginal gel was produced by Behvazan Pharmaceutical Company, Iran. It was re-filled in tubes that were similar to *H. perforatum* L. tubes.

### 2.7. Outcomes

The primary outcomes were success rate on Day 10 to 12 (the positive of one or none of Amsel's criteria) and the recurrence rate (positive of at least two out of four Amsel criteria) on days 30 to 35 after starting treatment<sup>[21]</sup>. Secondary outcomes included each of the patient complaints, treatment satisfaction level and adverse events.

### 2.8. Sample size and statistical analysis

The sample size was calculated using Stata software version 9.2 (Statasoft, Inc., Tula, USA). Considering 80% treatment rate with metronidazole, 5% one-tailed type I error, power of 80% and a 10% probable drop in the sample, the required sample size were calculated to be 80 for each group to detect a minimum difference of 20% in treatment rate.

Data analysis was performed using SPSS-version 13 software. *Chi*-square test, Fisher's exact test or binary logistic regression were used to compare the two groups in terms of frequency of initial positive response to treatment and recurrence rate, clinical and laboratory signs and patient complaints.

## 3. Results

### 3.1. Recruitment and follow-up

The participants were recruited from February to September 2012 and followed-up until October 2012. All participants attended the first follow-up on 10th–12th day. Fifteen out of 82 people in the *H. perforatum* group and 12 out of 80 in the metronidazole group who got oral metronidazole on 10th–12th day follow-up due to negative response to the treatment were excluded in the second follow up and one person from the *H. perforatum* group did not attend the

second follow up due to operation of low back disk hernia (She expressed her satisfaction with the treatment on the phone). Therefore, 66 participants in *H. perforatum* group and 67 participants in metronidazole group were assessed at the second follow-up on 30th–35th day.

### 3.2. Baseline characteristics

At the baseline, the reproductive and demographic characteristics, patient complaints, clinical observations and Amsel criteria of the two groups were similar. The mean age of the subjects was 33.7 (SD: 7.5) years and age at their first marriage was 19.4 (SD: 4.2). Almost four-fifth of the participants had primary or secondary education and 91% of them were housewives (Table 1). All participants in both groups had thin, homogeneous, grey and abundant discharge; vaginal pH greater than 4.5 and positive clue cell. All except four had positive whiff test (Table 2). The most common complaints of the subjects were malodorous vaginal discharge (93%), burning during intercourse (44%), Itching (38%) and lower abdominal pain (35%) (Table 3).

**Table 1**

Baseline characteristics of the participants by study groups.

Characteristics	<i>Hypericum</i> (n=82)	Metronidazole (n=80)
Age (years)	33.4±7.8	34.1±7.2
Age at marriage (years)	19.0±4.3	19.9±4.0
Educational level		
Illiterate	8 (10%)	8 (10%)
Primary (1–8 years)	37 (45%)	32 (40%)
Secondary (9–12 years)	32 (39%)	32 (40%)
University	5 (6%)	8 (10%)
Occupation (Housewife)	76 (93%)	71 (89%)
Family income less than expenses	11 (13.4%)	11 (13.8%)
Number of pregnancy	2.5 (1.9)	2.6 (2.0)
Number of vaginal delivery	1.6 (1.8)	1.4 (1.7)
History of abortion	25 (31%)	30 (38%)
Time of the last delivery (<3 years ago)	14 (19%)	18 (24%)
History of vaginal infection needed treatment	60 (73%)	56 (70%)

The data are given as *n* (%) unless otherwise is specified. There were no significant statistically difference between the groups in terms of any of the characteristics.

### 3.3. Outcomes

At the 10th–12th day follow up, the positive response to treatment was observed at 82% of *H. perforatum* group and 85% of the metronidazole group and there was no statistically significant difference between the groups [relative risk (RR) 0.9, 95% confidence interval (CI) 0.6 to 1.3, *P*=0.574]. Compared with metronidazole group, in *H. perforatum* group the frequency of homogenous discharge was significantly less (4% vs. 15%, *P*=0.013) and the frequency of pH>4.5 was more (60% vs. 36%, *P*=0.003). The two other Amsel criteria, the presence of clue cell and positive whiff test, were also significantly decreased in the both groups compared to the baseline but no significant difference was found between the groups in these criteria

**Table 2**

Rate of cure and recurrent of BV and each of Amsel criteria by the study groups.

Outcome variables	<i>Hypericum</i>			Metronidazole			Day 10–12		Day 30–35	
	Baseline (n=82)	Day 10–12 (n=82)	Day 30–35 (n=66)	Baseline (n= 80)	Day 10–12 (n= 80)	Day 30–35 (n= 68)	RR (95% CI)	P	RR (95% CI)	P
Primary outcomes										
Cure of BV	–	67 (82%)	–	–	68 (85%)	–	0.9 (0.6 to 1.3)	0.574	–	–
Recurrent of BV	–	–	6 (9%)	–	–	9 (13%)	–	–	0.8 (0.4 to 1.3)	0.447
Amsel criteria										
Homogenous discharge	82 (100%)	3 (4%)	1 (2%)	80 (100%)	12 (15%)	7 (10%)	0.4 (0.1 to 1.0)	0.013	0.2 (0.4 to 1.5)	0.062
Presence of clue cells	82 (100%)	22 (27%)	8 (12%)	80 (100%)	14 (18%)	8 (12%)	1.3 (0.9 to 1.8)	0.153	1.0 (0.6 to 1.7)	1.0
Positive whiff test	81 (99%)	8 (10%)	1 (2%)	77 (96%)	12 (15%)	2 (3%)	0.8 (0.4 to 1.3)	0.310	0.7 (0.1 to 3.4)	1.0
pH>4.5	82 (100%)	49 (60%)	21 (32%)	80 (100%)	29 (36%)	20 (29%)	1.6 (1.2 to 2.3)	0.003	1.1 (0.7 to 1.5)	0.852

RR: Relative risk; The data are given as n (%); Chi-squared or Fisher's exact test were used for the between groups comparisons.

**Table 3**

Frequency of patient complaints before and after intervention by the study groups.

Patient complaints	<i>Hypericum</i>			Metronidazole			Day 10–12		Day 30–35	
	Baseline (n=82)	Day 10–12 (n=82)	Day 30–35 (n=66)	Baseline (n=80)	Day 10–12 (n=80)	Day 30–35 (n=68)	OR (95% CI)	P	OR (95% CI)	P
Malodor discharge	77 (94%)	3 (4%)	2 (3%)	74 (93%)	2 (3%)	3 (4%)	1.5 (0.2 to 9.0)	0.684	0.7 (0.1 to 4.2)	0.676
Itching	33 (40%)	4 (5%)	8 (12%)	28 (35%)	13 (16%)	29 (43%)	0.3 (0.1 to 0.9)	0.026	0.2 (0.1 to 0.4)	<0.001
Dysuria	13 (16%)	0 (0%)	1 (2%)	7 (9%)	4 (5%)	0 (0%)	–	0.057*	–	0.493*
Burning	17 (21%)	3 (4%)	1 (2%)	14 (18%)	1 (1%)	0 (0%)	2.9 (0.3 to 28.4)	0.371	–	0.493*
Burning during intercourse	37 (45%)	6 (7%)	2 (3%)	34 (43%)	4 (5%)	0 (0%)	1.6 (0.4 to 6.1)	0.528	–	0.241*
Lower abdominal pain	29 (35%)	9 (11%)	4 (6%)	27 (34%)	8 (10%)	7 (10%)	1.1 (0.4 to 3.0)	0.873	0.6 (0.2 to 2.0)	0.370
Dyspareunia	11 (13%)	2 (2%)	2 (3%)	6 (8%)	0 (0%)	0 (0%)	–	0.497*	–	0.241*

OR: Odds ratio; The data are given as n (%); \*: For the between groups comparisons, Fisher's exact test was used for these ones and logistic regression adjusted for baseline values for the others.

(P=0.153, P=0.310, respectively) (Table 2).

At the 30th–35th day follow-up, the recurrence rate was 9% in *H. perforatum* group and 13% in metronidazole group (RR 0.8, 95% CI 0.4 to 1.3, P=0.447) among the participants with initial positive response to the treatment. There was no statistically significant difference between the groups regarding any of the Amsel criteria (P>0.05) (Table 2).

At the both follow-ups, there was no statistically significant difference (P>0.05) between the two groups regarding the patient complaints, except itching. Compared with metronidazole group, in *H. perforatum* group itching was significantly less at both the first follow-up (16% vs. 5%, P=0.026) and the second follow-up (43% vs. 12%, P<0.001) (Table 3).

Overall satisfaction with treatment was asked from the participants at the second follow-up with a 5-points Likert scale question. In *H. perforatum* group, 67% were very satisfied, 19% satisfied, 11% unsure and 3% dissatisfied. In metronidazole group, these percentages were 35%, 33%, 13% and 19%, respectively and Mann-Whitney U test indicated higher satisfaction in the *H. perforatum* group (P=0.001).

### 3.4. Side events

Eleven patients in the *H. perforatum* group had complaint of vaginal irritation during the first day of taking the gels. Nausea, vomiting and dizziness was reported by one and vaginal dryness by two of the participants of the intervention group. In the metronidazole group, vaginal burning during the drug use was reported by two

participants, nausea by two, vomiting by one, dizziness by two, vaginal dryness by four and metallic taste by one. In addition, 16 subjects in *H. perforatum* group and one participant in metronidazole group were very satisfied with the recovery of previous severe low back pain.

## 4. Discussion

To the best of our knowledge, this study is the first research performed regarding the effect of *H. perforatum* L. on BV in human. Its results revealed that effect of 3% vaginal gel of *H. perforatum* in treatment and prevention of disease relapse is similar to 0.75% metronidazole vaginal gel, it was well tolerated by people without having any significant side effects and most people were satisfied with it.

The results of this study are consistent with the results of the *in-vitro* studies assessed antibacterial effect of *Hypericum* extract in laboratory<sup>[17,23–27]</sup>.

In the study by Milosevic *et al.* the antimicrobial effect of the ethanol extract of *H. perforatum* L. was shown on the investigated ten types of bacterial<sup>[25]</sup>; eight types of Gram negative bacteria (*Pseudomonas fluorescens*, *Pseudomonas phaseolicola*, *Pseudomonas glycinea*, *Erwinia carotovora*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Agrobacterium tumefaciens*, *Azotobacter chroococcum*) and 2 types of Gram positive bacteria (*Bacillus mycoides*, *Bacillus subtilis*). Also in a study by Feyzioğlu *et al.*<sup>[26]</sup>, hypericin was effective on the *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228.

Furthermore, in a study by Yousuf *et al.*[23], antimicrobial activity of methanol extract of *H. perforatum* leaves was shown against all of the 6 bacteria under study (*Staphylococcus epidermidis* MTCC– 435, *Bacillus subtilis* MTCC–441, *Proteus vulgaris* MTCC–321, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*).

Also, in studies by Zdunić *et al.*[28], and Paterniti *et al.*[29], the anti-inflammatory effect of *H. perforatum* L. extract and in Abdel-Salam's study[30], its anti-edematogenic and analgesic effects on rats have been shown which might be related to the antimicrobial effect of this plant.

Occurring no serious side event in *H. perforatum* vaginal gel users in this study is similar to results of previous studies using other forms of this herb on human subjects affected with other disorders[14–16].

Blinding the participants and the people involved in the recruitment, data collection, outcome assessment and data analysis, no attrition in the first follow-up and follow-up of all but one participant in the second stage can be mentioned as strengths of this study. Larger samples size, compared to most other studies on bacterial vaginosis, is another strength point of this study.

Not using Nugent method as the golden standard for diagnosis and treatment, as well as short follow-up period (only 1 month) could be mentioned as the study limitations. Therefore, we suggest other studies in this area with longer follow-up and using diagnosis methods with higher sensitivity and specificity in other settings.

Responses to the plant may occur with delay in some people. However, it was not possible to assess the potential delayed effect. Due to ethical issues (no previous information on the efficacy of the treatment on human beings), in this study all the participant who did not have positive response on 10th–12th days follow-up (27 of 162 participants) were treated with oral metronidazole without knowing the participants' group. According to the results of this study about of similar success rate in the two groups, re-treatment could be delayed until the following month in subsequent studies to determine the potential delayed effect.

In conclusion, the findings of this study showed that 3% *H. perforatum* vaginal gel is effective in treatment of the patients suffering from bacterial vaginosis and decreasing their complaints. Given the high acceptance by patients and lack of any serious side effects, it seems that this gel could be used as an effective treatment option in bacterial vaginosis, especially in drug resistance cases and also for those interested in herbal therapies. However, further studies are needed for its public use.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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midwifery of Tabriz International University of Medical Sciences (Aras) with code of 181108. The project was approved by ethics committee of Tabriz University of Medical Sciences (code: 9060, date: 12.25.2011) and registered in Iranian registry controlled trial with IRCT201112063706N9 code (<http://apps.who.int/trialsearch/trial.aspx?trialid=IRCT201112063706N9>). The authors hereby express their thanks to the women participated at this study and the midwives who were referred as the study patients.

## Comments

### Background

Bacterial vaginosis is the most common cause of vaginal discharge in women. It can cause bothersome symptoms, and also increase the risk of acquiring serious sexually transmitted infections. The metronidazole is one of the most effective treatments. Because of the side effects of this antibiotic, we need an alternative.

### Research frontiers

This research focuses on the study of the effect of *H. perforatum* compared with metronidazole in bacterial vaginosis. This work was carried out on 162 married women 18–49 years old. The whiff test is the main test used to determine bacterial vaginosis.

### Related reports

Russo *et al.* (2013) have published a review article about *H. perforatum* entitled Pharmacokinetic, mechanism of action, tolerability, and clinical drug–drug interactions. *H. perforatum* extracts appear to be well tolerated. However, on the basis of our knowledge, there are few researches that are interested in this kind of study on bacterial vaginosis (Simbar *et al.*, 2008).

### Innovations and breakthroughs

Antibiotics used against bacterial vaginosis are very limited. A few antibiotic remedies are routinely used and include: metronidazole, clindamycin, or tinidazole. It requires very effective and safe therapeutic alternatives. The innovation in this paper is the use of *H. perforatum* extracts as an alternative against bacterial vaginosis (*in vivo*).

### Applications

We can apply this natural product (*H. perforatum* vaginal gel) in pharmaceutical industry and in the treatment of bacterial vaginosis.

### Peer review

This work represents an important study. These researchers have tested *H. perforatum* vaginal gel on 162 married women 18–49 years old. They found that this gel exhibits a very interesting activity against the bacteria responsible for bacterial vaginosis. In all the cases, the originality of this work gave us a great satisfaction on the

importance of this study in a number of biological and medicine fields.

## References

- [1] Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. *Am J Obstet Gynecol* 2013; **209**: 505–523.
- [2] Soper DE. Gentiourinary infectious and sexually transmitted diseases. In: Berek JS, editor. *Berek & Novak's gynecology*. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2007, p. 558–559.
- [3] Oduyebo OO, Anorlu RI, Ogunsola FT. The effects of antimicrobial therapy on bacterial vaginosis in non-pregnant women. *Cochrane Database Syst Rev* 2009; doi: 10.1002/14651858.CD006055.pub2.
- [4] Sobel JD. Bacterial vaginosis. USA: UpToDate, Inc.; 2014. [Online] Available from: [http://www.uptodate.com/contents/bacterial-vaginosis?source=search\\_result&search=bacterial+vaginosis&selectedTitle=1~150](http://www.uptodate.com/contents/bacterial-vaginosis?source=search_result&search=bacterial+vaginosis&selectedTitle=1~150) [Accessed on 8th May, 2014]
- [5] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. Diseases characterized by vaginal discharge. Atlanta: Centers for Disease Control and Prevention; 2010. [Online] Available from: <http://www.cdc.gov/std/treatment/2010/vaginal-discharge.htm#a1>. [Accessed on 8th May, 2014]
- [6] Nagaraja P. Antibiotic resistance of *Gardnerella vaginalis* in recurrent bacterial vaginosis. *Indian J Med Microbiol* 2008; **26**: 155–157.
- [7] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol* 2014; **4**: 177.
- [8] Simbar M, Azarbad Z, Mojab F, Majd HA. A comparative study of the therapeutic effects of the *Zataria multiflora* vaginal cream and metronidazole vaginal gel on bacterial vaginosis. *Phytomedicine* 2008; **15**(12): 1025–1031.
- [9] Mašković PZ, Mladenović JD, Cvijović MS, Aćamović-Đoković G, Solujić SR, Radojković MM. Phenolic content, antioxidant and antifungal activities of acetonic, ethanolic and petroleum ether extracts of *Hypericum perforatum* L. *Hemijska industrija* 2011; **65**(2): 159–164.
- [10] WHO. *WHO monographs on selected medicinal plants*. Geneva: World Health Organization; 1999, p. 149–171.
- [11] Barnes J, Anderson LA, Phillipson JD. St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *J Pharm Pharmacol* 2001; **53**(5): 583–600.
- [12] Goldaru. Perforan coated tablet. Iran: Goldaru. [Online] Available from: <http://goldaru-co.com/index.php/en/neurology-and-psychiatry/perforan-coated-tablet> [Accessed on 28th May, 2013]
- [13] Esensa d.o.o. Company. Hypervag® intim gel. Belgrade, Serbia: Esensa d.o.o. Company. [Online] Available from: <http://en.esensa.rs/proizvodi/Hypervag%C2%AE-Intim-Gel/9> [Accessed on 28th May, 2013]
- [14] Läuchli S, Hafner J, Wehrmann C, French LE, Hunziker T. Post-surgical scalp wounds with exposed bone treated with a plant-derived wound therapeutic. *J Wound Care* 2012; **21**(5): 228, 230, 232–233.
- [15] Canning S, Waterman M, Orsi N, Ayres J, Simpson N, Dye L. The efficacy of *Hypericum perforatum* (St John's wort) for the treatment of premenstrual syndrome: a randomized, double-blind, placebo-controlled trial. *CNS Drugs* 2010; **24**(3): 207–225.
- [16] Samadi S, Khadivzadeh T, Emami A, Moosavi NS, Tafaghodi M, Behnam HR. The effect of *Hypericum perforatum* on the wound healing and scar of cesarean. *J Altern Complement Med* 2010; **16**(1): 113–117.
- [17] Meral GE, Karabay NU. *In vitro* antibacterial activities of three *Hypericum* species from West Anatolia. *Turkish Electron J Biothechnol* 2002; (Special Issue): 6–10.
- [18] Franklin G, Conceição LFR, Kombrink E, Dias AC. Xanthone biosynthesis in *Hypericum perforatum* cells provides antioxidant and antimicrobial protection upon biotic stress. *Phytochemistry* 2009; **70**: 60–68.
- [19] Saddiqe Z, Naeem I, Maimoona A. A review of the antibacterial activity of *Hypericum perforatum* L. *J Ethnopharmacol* 2010; **131**(3): 511–521.
- [20] Udayalaxmi, Bhat G, Kotigadde S, Shenoy S. Comparison of the methods of diagnosis of bacterial vaginosis. *J Clin Diagn Res* 2011; **5**(3): 498–501.
- [21] Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983; **74**(1): 14–22.
- [22] Hemmati AA, Rashidi I, Jafari M. Promotion of wound healing by *Hypericum perforatum* extract in rabbit. *Jundishapur J Nat Pharm Prod* 2007; **2**(2): 78–86.
- [23] Yousuf M, Aslam K, Wani BA, Aslam N, Dar NA, Nawchoo IA. *In vitro* antibacterial activity and phytochemical studies of methanolic extract of leaves of *Hypericum perforatum* L. growing wild in Kashmir Himalaya. *Asian J Plant Sci Res* 2012; **2**(4): 414–420.
- [24] Alireza M. Antimicrobial activity and chemical composition of essential oils of four *Hypericum* from Khorasan, Iran. *JMPR* 2012; **6**(12): 2478–2487.
- [25] Milosevic T, Solugic S, Sukdolac S. *In vitro* study of ethanolic extract of *Hypericum perforatum* L. on growth and sporulation of some bacteria and fungi. *Turkish J Biol* 2007; **31**: 237–241.
- [26] Feyzioğlu B, Demircili MH, Özdemir M, Doğan M, Baykan M, Baysal B. Antibacterial effect of hypericin. *Afr J Microbiol Res* 2013; **7**(11): 979–982.
- [27] Borchardt JR, Wyse DL, Sheaffer CC, Kauppi KL, Fulcher RG, Ehlke NJ, et al. Antimicrobial activity of native and naturalized plants of Minnesota and Wisconsin. *J Med Plants Res* 2008; **2**(5): 98–110.
- [28] Zdunić G, Godevac D, Milenković M, Vucićević D, Savikin K, Menković N, et al. Evaluation of *Hypericum perforatum* oil extracts for an anti-inflammatory and gastroprotective activity in rats. *Phytother Res* 2009; **23**(11): 1559–1564.
- [29] Paterniti I, Briguglio E, Mazzon E, Galuppo M, Oteri G, Cordasco G, et al. Effects of *Hypericum perforatum*, in a rodent model of periodontitis. *BMC Complement Altern Med* 2010; **10**: 73.
- [30] Abdel-Salam OM. Anti-inflammatory, antinociceptive, and gastric effects of *Hypericum perforatum* in rats. *Sci World J* 2005; **5**: 586–595.