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ORIGINAL ARTICLE

A randomized trial comparing a polyherbal pessary (a complementary and alternative medicine) with Ginlac-V pessary (containing clotrimazole, tinidazole and lactobacilli) for treatment of women with symptomatic vaginal discharge

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Abstract
Background Most women with abnormal vaginal discharge have infection due to candida species, bacterial vaginosis or trichomoniasis and often seek treatment without laboratory confirmation. In this context, a single agent effective against these infections would be useful.
Aim To compare the efficacy of two such agents: Praneem polyherbal pessary; a complementary and alternative medicine (CAM) with Ginlac-V pessary (containing clotrimazole, tinidazole and lactobacilli) for treatment of abnormal vaginal discharge.
Settings and design A randomized study in a tertiary care hospital in North India. Methods: One hundred women were randomized for treatment with either of the two pessaries. Clinical examination and laboratory evaluation was done before and after treatment.
Statistical analysis Mc-Nemar test and Chi-square test.
Results Overall, 82% (82/100) reported symptomatic relief; 78% (39/50) with Praneem and 86% (43/50) with Ginlac-V. Only 36% (18/50 in each group) had laboratory-confirmed infection; 18% (18/100) candidosis, 17% (17/100) bacterial vaginosis, 1% (1/100) both; none had trichomoniasis. Among these, symptomatic improvement was seen in 72% (13/18) and laboratory cure in 78% (14/18) with Praneem; symptomatic improvement in 78% (14/18) and laboratory cure in 78% (14/18) with Ginlac-V. Clinical or laboratory criteria could assess treatment efficacy equally. Neither drug was efficacious in candidosis. Ginlac-V was efficacious in bacterial vaginosis (100%) and though Praneem showed a similar trend, it was not statistically significant. Vaginal irritation was more frequent with Praneem (16% vs 4% with Ginlac-V).
Conclusion Both Praneem and Ginlac-V provided symptomatic relief in most of the women. Either clinical or laboratory criteria could assess treatment efficacy of both drugs. Ginlac-V was efficacious for treating bacterial vaginosis.

Keywords Symptomatic vaginal discharge · Bacterial vaginosis · Candida vaginitis · Complementary and alternative medicine (CAM) · Praneem polyherbal pessary · Trichomoniasis · Vaginal candidosis

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Introduction

Women commonly seek treatment for symptomatic vaginal discharge. Fifteen percent women attending a family planning clinic had vaginal infection with laboratory confirmation in 70%. Trichomoniasis, candidosis and bacterial vaginosis were similarly prevalent (22–26%) and account for most cases of vaginal infection [1]. Others found bacterial vaginosis to be the most prevalent among adults (23.8%) followed by *Candida* species (17.8%), *Streptococcus agalactiae* (5.6%) and *Trichomonas vaginalis* (2.4%). In 50.3%, no microorganism was detected. Among adolescents, *Candida* species were the commonest (29.7%), followed by bacterial vaginosis (17.8%), *Streptococcus agalactiae* (3.6%) and *Trichomonas vaginalis* (2.4%). No microorganism was isolated in 46.4% [2].

Oral tinidazole and metronidazole are effective in trichomoniasis and bacterial vaginosis. Vaginal metronidazole and tinidazole are effective in bacterial vaginosis [3,4]. Vaginal clotrimazole and oral fluconazole are effective in vaginal candidosis [4]. Lactobacilli used orally or vaginally are effective for prophylaxis of candidosis [5,6]. In the absence of a microbiological evaluation of the causative organism, a combination of these drugs is prescribed. In this context, a therapeutic agent that treats all the common vaginal infections would be of utility.

Praneem, a complementary and alternative medicine (CAM) is a polyherbal vaginal pessary, which was developed using purified extracts of neem *(Azadirachta indica)*, saponins and mentha-citrata oil. This formulation inhibits a wide range of microbial and viral pathogens of the genital tract, proven by in-vitro and animal studies including *Candida albicans, Candida krusei, Candida tropicalis, Neisseria gonorrhoeae, Escherichia coli, Herpes simplex, Chlamydia trachomatis* and HIV-1 [7,8]. This formulation exercises local effect and is not expected to cure deep-seated and systemic infections like gonorrhoea, chlamydia and HIV; nonetheless, its strong microbicidal action on these would offer protection against transmission of these sexually. Multi-centric Phase-1 studies found Praneem to exhibit no clinical, biochemical and haematological side effects in healthy women volunteers [9,10].

Ginlac-V pessary (Rapross Pharmaceuticals Ltd., Delhi) contains tinidazole 500 mg, clotrimazole 200 mg and lactobacilli. Clotrimazole, lactobacilli and tinidazole used vaginally are efficient for treatment of candidosis and bacterial vaginosis; however, the efficacy of vaginal tinidazole in trichomoniasis has not been widely evaluated, though simultaneous administration of oral and vaginal tinidazole was effective in a case of oral metronidazole and oral tinidazole-resistant trichomonas vaginitis [11].

This study was planned to determine the efficacy of Praneem pessary for the treatment of women with symptomatic vaginal discharge in view of its proposed therapeutic action against multiple organisms and compare its efficacy with Ginlac-V pessary, which offers coverage against the three common vaginal infections.

Materials and methods

This prospective randomized study was conducted in a tertiary care hospital in North India after obtaining ethical clearance from the hospital's ethical committee. One hundred and eighteen married women in the reproductive age group (18–45 years) with good general health, who presented to the Gynaecology outpatients' department of the Hospital with complaints of abnormal vaginal discharge between April 2002 and November 2003 were counselled

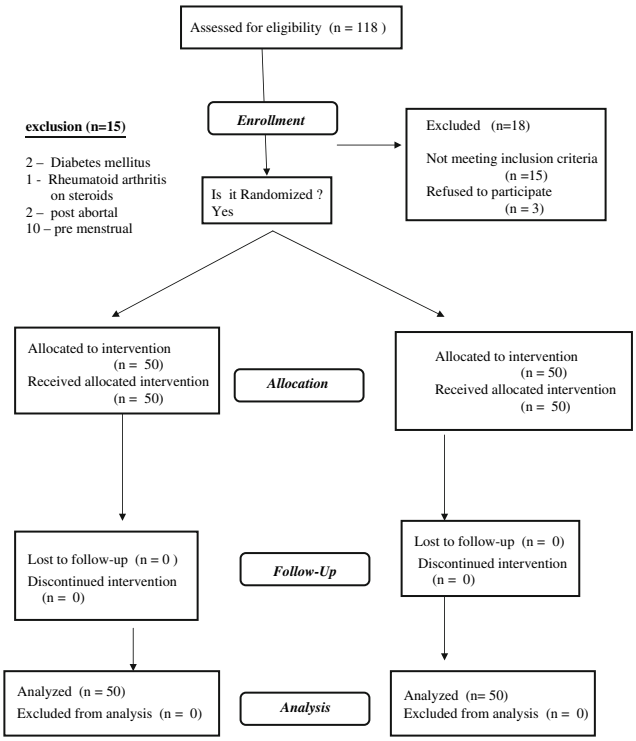

Fig. 1 The Consort—flowchart

for this study of which 100 were finally selected (Fig. 1). The vaginal discharge was considered to be abnormal if the women reported it to be excessive (requiring use of panty liners), thin homogenous or frothy or curdy-white, staining the undergarments and associated with unpleasant odour or pruritis. The presence of vaginal discharge was confirmed by a speculum examination. Women who were pregnant, <6 weeks post-abortal or post-partum, diabetic, immunocompromised, on chronic drug therapy like steroids, with abnormalities of lower genital tract (which would interfere with placement of pessary), pelvic inflammatory disease and known or suspected genital malignancy were excluded. A written informed consent was obtained from all women before recruitment. The primary outcome was relief in symptoms and the secondary outcomes were cure of laboratory proven infection, comparison of symptomatic relief and laboratory cure in women with laboratory evidence of infection and efficacy in treatment of individual vaginal infections.

General and pelvic examinations were performed and samples for laboratory evaluation were collected. The women were randomized into two groups using a computer-generated randomization table. The study was randomized but not blinded, as the number of pessaries was different in the two groups. The women recruited in the study as well as the clinicians knew which women belonged to Group-1 or Group -2. Group-1 was given seven Praneem pessaries and group 2 was given four Ginlac-V pessaries. They were instructed to place one pessary in the

vagina with soap-washed fingers daily at bedtime and refrain from intercourse till follow-up, 3–5 days after the last pessary. At follow-up, examination was performed and all the initial sample collection was repeated. Treatment compliance, persistence or relief of symptoms and side effects were noted. Those with inadequate relief were treated further by the attending physician.

Collection and processing of samples

Using a sterile Cusco's speculum, vaginal discharge was collected from the posterior fornix onto sterile swabs for culture of yeasts, *Gardnerella vaginalis, Mobiluncus* and other anaerobes, which were transported to the laboratory within 6 h. A cervical cytology smear, a wet-mount smear for *Trichomonas vaginalis*, yeasts, clue cells and pus cells and an air-dried smear for Gram staining were also prepared.

PAP smear: Cytological examination was done as per Bethesda classification [12].

Vaginal pH test: A vaginal swab from the posterior fornix was touched onto pH indicator paper strips with a range 4.0–10.0 (Merck, Germany).

Wet-mount smear: A drop of vaginal discharge was placed on two glass slides, diluted with a drop of 0.9% saline, covered with a cover slip and examined under high power. *Trichomonas vaginalis* are clear, pear-shaped organisms about the size of a pus cell, with four anterior flagellae, an axostyle and a characteristic jerky motility. In bacterial vaginosis, the number of epithelial cells/HPF (high-power field) exceeds the number of leukocytes; there is appearance of clue cells and a reduction in the number of lactobacilli (Amsel's criteria) [13]. A VIP (vaginal identification of pathogens) wet preparation with 0.2% crystal violet stain was done on one slide to identify clue cells. To improve visualization of yeast, 10% KOH was added to the other slide to disrupt cellular material that might obscure them.

Amine test: This was done by addition of 10% KOH onto the wet smear to assess for the typical fishy odour in bacterial vaginosis [13].

Gram's staining and Nugent's scoring: An air-dried smear was stained using Gram's stain and Nugent's criteria were used for scoring for bacterial vaginosis [14].

Culture: The vaginal swabs were inoculated on blood agar and human blood agar for growth of *Gardnerella vaginalis* and on Sabouraud's dextrose agar (SDA) for growth of yeasts [15,16]. Blood agar and SDA were incubated at 37°C for 48 h. Good quality reagents and chemicals (Difco or Glaxo company) were used. The other blood agar plate was kept in anaerobic conditions (10% CO_2 at 37°C for 24 h) for the growth of Mobiluncus species and other anaerobes. The organisms were identified according to colony morphology and standard methods [15,16].

Efficacy of the treatment was assessed during follow-up based on symptomatic relief, pelvic examination and laboratory investigations in the form of normal wet smear, pH of 4.0, a normal Nugent's score and absence of any growth on cultures. Absence of abnormal vaginal discharge in women reporting relief in symptoms was confirmed by a speculum examination.

Statistical analysis was done by the Chi square test to compare the clinical cure rates of the two drugs and the McNemar test to evaluate the same patients for clinical cure and laboratory cure. Assuming a clinical cure rate of 80%, a sample size of 46 women in each group would be able to detect a 30% difference in the clinical cure rates of Ginlac-V and Praneem vaginal pessaries.

Results

Baseline parameters

The mean age was similar; 29.2 years (range 20–45) in group 1 and 28.8 years (range 20–44) in group 2 ($P = 0.71$). The majority was multiparous; only 4% (2/50) in group 1 and 16% (8/50) in group 2 were nulliparous. The parity distribution was similar ($P > 0.1$). Type of contraceptive used was similar ($P > 0.1$); condom (14% or 7/50 in group 1 and 18% or 9/50 in group 2); IUCD (44% or 22/50 in group 1 and 28% or 14/50 in group 2); sterilization (18% or 9/50 in group 1 and 24% or 12/50 in group 2); hormonal (4% or 2/50 in each group) and none (20% or 10/50 in group 1 and 26% or 13/50 in group 2). PAP smear was normal in 92% and inflammatory in 8%.

Laboratory evidence of infection

The presence of vaginal infection was confirmed in 36% (36/100) women by laboratory evaluation; 18% (18/100) had bacterial vaginosis, 17% (17/100) had candidosis, 1% (1/100) had mixed infection with both (she was in group-1) and none had trichomoniasis. The women with laboratory evidence of infection were distributed equally in the two groups. Each group had 18/50 women with laboratory evidence of infection.

Comparison of symptomatic improvement and laboratory cure

Symptomatic relief was felt by 82% overall; 39/50 (78%) with Praneem and 43/50 (86%) with Ginlac-V. The difference in the efficacy of Praneem or Ginlac-V to provide symptomatic relief was not significant ($P > 0.05$; Table 1). Table 2 shows the comparison of symptomatic relief and

Table 1 Symptomatic relief in women with or without laboratory evidence of infection

Laboratory evidence of infection	Symptomatic relief with Praneem (group 1)	Symptomatic relief with Ginlac-V (group 2)	Total ($n = 100$)	P value (Chi-square)
Yes ($n = 36$)	13/18 (72%)	14/18 (78%)	27/36 (75%)	$P = 1.00$, NS
No ($n = 64$)	26/32 (81%)	29/32 (91%)	55/64 (86%)	$P = 0.47$, NS
Total	39/50 (78%)	43/50 (86%)	82/100 (82%)	$P = 0.43$, NS

Table 2 Comparison of symptomatic relief versus laboratory cure in women with laboratory evidence of infection (total = 36, Group-1, $n = 18$ and Group-2, $n = 18$)

Laboratory cure	Symptomatic relief		Total	Mc-Nemar test
	Yes	No		
Group-1 (Praneem, $n = 18$)				
Yes	11 (61%)	3	14 (78%)	Chi-square = 0
No	2	2	4	$P = 0.53$, NS
Total	13 (72%)	5	18	
Group-2 (Ginlac-V, $n = 18$)				
Yes	12 (67%)	2	14 (78%)	Chi-square = 0.25
No	2	2	4	$P = 0.197$, NS
Total	14 (78%)	4	18	

Table 3 Efficacy of treatment in vaginal candidosis

Before treatment	After treatment			Mc-Nemar test
	Yes	No		
Praneem (Group-1) in vaginal candidosis ($n = 8$)				
Yes	8	0	8	Chi-square = 1.5
No	42	3	39	$P = 0.227$, NS
Total	50	3	47	
Ginlac-V (Group-2) in vaginal candidosis ($n = 10$)				
Yes	10	4	6	Chi-square = 1.1
No	40	2	38	$P = 0.289$, NS
Total	50	6	44	

laboratory cure in women with laboratory evidence of infection. In group 1 (Praneem), 14/18 (78%) were cured by laboratory parameters while 13/18 (72%) had symptomatic relief. Concurrent symptomatic improvement and laboratory cure were found in 11/18 (61%). In group 2 (Ginlac-V), 14/18 (78%) were cured by laboratory parameters while 14/18 (78%) had symptomatic relief. Concurrent symptomatic improvement and laboratory cure were found in 12/18 (67%). In both groups, either clinical or laboratory criteria could be used to assess treatment efficacy (McNemar test, $P > 0.05$).

Vaginal candidiasis

Eight out of fifty women (16%) in group-1 (Praneem) and ten out of fifty (20%) in group-2 (Ginlac-V) had laboratory evidence of candidosis based on culture. In group-1, all eight were cured (7 = Candida albicans, 1 = Candida tropicalis) but three initially un-infected women had candidiasis on follow-up (Table 3). In group-2 (9 = Candida albicans, 1 = Torulopsis glabrata), six out of ten (60%) were cured, whereas four (including the one with Torulopsis glabrata infection) had persistence of infection. Two initially un-infected women had candidosis at follow-up. Neither drug was found to be significantly efficacious in the treatment of candidosis. But if women who were initially un-infected and showed post-treatment laboratory evidence of infection were excluded from analysis, then both drugs were found to be significantly effective to treat candidosis. The mean pre-treatment vaginal pH in those with candidosis was 4.63 in group 1 and 4.70 in group 2. It was unaltered by use of either drug, irrespective of cure. One woman in group-1 (Praneem) had both bacterial vaginosis and candidosis. She was cured of candidosis but not of bacterial vaginosis.

Bacterial vaginosis

Eleven out of fifty women (22%) in group-1 and eight out of fifty (16%) in group-2 had bacterial vaginosis diagnosed by Amsel's criteria, Nugent's score 7–10 or culture (two or more criteria positive). Praneem cured seven out of eleven, but one initially un-infected woman had infection at follow-up (Table 4). Though Praneem showed a trend towards being efficacious in the treatment of bacterial vaginosis, this was not found to be statistically significant. Ginlac-V was significantly efficacious in treating bacterial vaginosis; all eight were cured and no previously un-infected woman had infection at follow-up. The mean pre-treatment vaginal pH in women with bacterial vaginosis was 5.18 in group 1 and 5.06 in group 2. After treatment with Praneem the pH fell significantly from 5.18 to 4.36 ($P < 0.01$) in those cured of infection while it remained high (5.10) in those who persisted with infection. With use of Ginlac-V, the pH fell significantly from 5.06 to 4.63 ($P < 0.05$).

Side effects

No major side effects or interruption of treatment was seen in either group. Burning sensation in the vagina was felt by

Table 4 Efficacy of treatment in bacterial vaginosis

Before treatment		After treatment		Mc-Nemar test
		Yes	No	
Praneem (Group-1) in bacterial vaginosis (n = 11)				
Yes	11	4	7	Chi-square = 3.125
No	39	1	38	P = 0.07, NS
Total	50	5	45	
Ginlac-V (Group-2) in bacterial vaginosis (n = 8)				
Yes	8	0	8	Chi-square = 6.1
No	42	0	42	P = 0.007, significant
Total	50	0	50	

eight out of fifty (16%) with Praneem and two out of fifty (4%) with Ginlac-V. One woman (2%) had staining of underclothes with Praneem and 2 (4%) with Ginlac-V.

Discussion

When treatment for symptomatic vaginal discharge is prescribed without laboratory confirmation, it is based upon the clinical diagnosis of a particular infection (curdy white discharge in candidosis, frothy green in trichomoniasis and homogenous thin, grey-white adherent to vagina in bacterial vaginosis). Usually a formulation effective against all these infections is preferred. A combination pessary of clotrimazole, tinidazole and lactobacilli has therapeutic effect against these infections. Oral fluconazole and metronidazole are effective but have some side effects and vaginal therapy is preferred by women [17]. Experience with CAM therapy for bacterial vaginosis and vaginal candidosis is limited but a recent review has shown it to be beneficial [18]. Praneem polyherbal pessary is a CAM formulation, which has growth inhibitory effects on various microbial agents [7,8]. Therefore, its potential as a broad-spectrum therapeutic agent for vaginitis was explored.

Both pessaries were similarly effective in relieving symptoms. An important observation of this study is that in women with laboratory-proven infection, good correlation was found between symptomatic relief and laboratory cure.

Most women in this study were IUCD users; only 4% used hormonal contraception. This is in contrast to 64% women using hormonal contraception in a similar study [19]. The incidence of candidosis (18%) was similar to the 15 or 20% reported in literature [20,21]. A higher incidence (26%) is reported among STD patients [21]. Of the 18/100 women with candidosis, 16(88.9%) had *Candida albicans*, 1(5.6%) had *Candida tropicalis* and 1(5.6%) had *Torulopsis glabrata*. A similar incidence of *Candida albicans* (81%) but higher incidence of *Torulopsis glabrata* (16%) among STD patients is reported, possibly because such patients are likely to have resistant infections [22]. With Praneem, all eight (100%) were cured (7 = *Candida albicans*, 1 = *Candida tropicalis*) but three initially un-infected women had candidosis on follow-up. With Ginlac-V, six out of ten (60%) were cured, whereas four (including *Torulopsis glabrata*) had persistent infection. Two initially un-infected women had candidosis at follow-up. Neither drug was significantly efficacious in the treatment of candidosis, rather, the presence of post-treatment infection in previously un-infected women implies that they got infected despite therapy. Treatment of uncomplicated vaginal candidosis with topical azoles results in symptomatic relief and negative cultures in 80–90% [3,23]. Sobel et al. found a single oral 150 mg dose of fluconazole resulting in a cure rate of 76% as compared to 72% with 6–7 days' treatment with 100 mg vaginal clotrimazole [24]. The cure rate with either Praneem or Ginlac-V in candidosis was lower than reported rates of oral or topical azoles. It is possible that topical use of a lower dose of clotrimazole for a longer duration (100 mg for 7 days, CDC [4]) may be more efficacious than a higher dose for a shorter duration as in Ginlac-V (200 mg for 4 days). The mean pre-treatment vaginal pH in those with candidosis was lower than in those with bacterial vaginosis as expected and was unaltered by either drug, irrespective of cure.

In bacterial vaginosis, normal floras are replaced with *Peptococci*, *Bacteroides*, *Mycoplasma hominis*, *Gardnerella vaginalis* and anaerobes like *Mobiluncus* [25,26]. Its incidence in the present study (19%) is higher than the reported incidence of 9–12% [27–29]. With Praneem, seven out of eleven (64%) were cured but there was one new infection among those previously un-infected. Though Praneem showed a trend towards efficacy to treat bacterial vaginosis, it was statistically not significant. Ginlac-V was significantly efficacious and cured all eight (100%), with no new infections. The reported cure rates of bacterial vaginosis are 84–97% with oral metronidazole and tinidazole and 75% with vaginal metronidazole [4]. The better efficacy of Ginlac-V may also be due to the acidic pH provided by lactobacilli, which is unfavourable for the growth of organisms causing bacterial vaginosis. The use of Ginlac-V resulted in a significant fall in pH in all women with bacterial vaginosis (all were cured), whereas use of Praneem showed a significant fall in pH in only those who were cured.

In the present study, using only wet mount (60% sensitivity) as a diagnostic modality, no woman was found to have trichomoniasis. Using wet mount plus culture, trichomoniasis was found in 10–15% [30,31]. Culturing for *trichomonas vaginalis* may have diagnosed some cases. Also, those 64% women who did not demonstrate laboratory evidence of infection may be having aerobic vaginitis where there is reduction of lactobacilli and increase in aerobes like

Streptococcus agalactiae and *Eisherichia coli*, which were not looked for [32].

Vaginal irritation was more with Praneem than Ginlac-V but it was mild and did not deter pessary use. CAM therapies like vaginal boric acid application is reported to cause burning sensation in similar number of women using Praneem (4%) [18]. Tea-tree oil occasionally causes allergic dermatitis. Clotrimazole may cause irritation with topical use [33].

Summarizing, both Praneem and Ginlac-V were similarly effective in relieving symptoms in about four-fifths of women with abnormal vaginal discharge, though only Ginlac-V was significantly effective in the treatment of bacterial vaginosis. Symptomatic relief may be used to predict cure as accurately as laboratory confirmation. In an earlier study, intra-vaginal Praneem cream was efficacious against *Chlamydia*, partially effective against bacterial vaginosis, but not against candidosis or trichomoniasis [34]. Praneem may prove to be an alternative to the standard therapeutic agents for abnormal vaginal discharge but its side effect of vaginal irritation needs to be reduced. However, more studies are needed to improve the cure rates and these could investigate whether more prolonged use of either drug would improve treatment efficacy.

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