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Research Article

CLINICAL EVALUATION OF COMPARATIVE AND COMBINED EFFECT OF POLYHERBAL MICROBICIDE (BASANT) AND SELECTED PROBIOTICS IN THE TREATMENT OF RECCURRENT VAGINOSIS - A PHASE II PLACEBO CONTROLLED TRIAL

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ABSTRACT

Background: Vaginosis is a widely prevalent syndrome in women. Treatment with one or more antibiotics cures invariably the infections. However recurrence is frequent and pH of the vagina is not always restored to the normal acidic range. This trial was conducted to determine whether a Polyherbal Microbicide BASANT or three selected strains of Probiotics Lactobacilli alone or the two in combination can regress Vaginosis, bring down the vaginal pH to acidic range and restore healthy vagina with colonised *Lactobacilli*.

Methods: Women suffering from recurrent episodes of vaginosis were given capsules of either BASANT, Probiotics, Combination of the two or Placebo capsules for insertion in vagina for 7 nights. On day 10, Pelvic examination, pH, Whiff test, Gram stain for Clue cells and swab taken for culture of lactobacilli.

Results: BASANT regressed Vaginosis in 14/ 20, Probiotics in 13/20, Combination of the two in 19/ 20 women and Placebo in 1/ 20 women.

Conclusion: While both Polyherbal microbicide BASANT and the three strains of the lactobacilli cured Vaginosis in 65 to 70% of women, the combination was highly effective in curing 95% of women.

KEYWORDS: Vaginosis, BASANT, Pro-vag-Health Probiotics, pH, Colonisation.

INTRODUCTION

Vaginosis is a widely prevalent syndrome. As per an Indian Council of Medical Research (ICMR) study, the prevalence of this syndrome is about 30% in women attending the antenatal clinics in urban Delhi[1]. Bang et al[2] reported its incidence to be 50% in rural Maharashtra. Its incidence was 80% in slums of Delhi in a survey conducted by us along with Safdarjung Hospital and Population Foundation of India[3]. It is frequently accompanied by itching and malodour. The present treatment for Bacterial vaginosis employs various antibiotics which have different spectrum of activity with a cure rate at 4 weeks of 60%–70%[4].In another study of treatment with Metronidazole, 58% women had a recurrence of Bacterial vaginosis[5].Invariably this uncomfortable syndrome is accompanied by the pH of vagina rising above 5, indicative of the paucity or absence of lactic acid producing strains of *Lactobacilli.*

A Polyherbal Microbicide BASANT was developed which has inhibitory action on a wide spectrum of genital pathogens. BASANT inhibits

the growth of WHO strains and clinical isolates of *Neisseria gonorrhoeae*, including those resistant to Penicillin, Tetracycline, Nalidixic acid and Ciprofloxacin[6]. It has pronounced inhibitory action against *Candida glabrata*, *Candida albicans* and *Candida tropicalis* isolated from women with vulvovaginal candidiasis, including three isolates resistant to azole drugs and amphotericin. BASANT has a high virucidal action against human immunodeficiency virus. It inhibits also the entry in Hela cells of HPV16[6]. It has inhibitory action on free as well as cell-infected *Chlamydia trachomatis*[7]. BASANT was found to be totally safe according to pre-clinical toxicology carried out as per USFDA recommendations on rabbit vagina after application for 7 consecutive days or twice daily for 3 weeks.

We reported previously the isolation of 80 strains of lactobacilli from healthy vagina[8].Amongst these were strains which were either high or low producers of lactic acid[9].There was marked variation in their ability to make and secrete in quantitative terms hydrogen peroxide; their degree of hydrophobicity and other metabolic characteristics. Three strains namely TRF#36 strain of *Lactobacillus fermentum*, TRF#8 strain of *L. gasseri*, and TRF#30 strain of *L. salivarius* were selected on basis of their ability to make large amount of lactic acid, high hydrophobicity, positivity for arginine deiminase, thereby competence to prevent the formation of foul odour derivatives.

With the permission of The Drugs Controller General of India and with the approval of Institutional Ethics Committees, trials were conducted in two Institutions to determine the ability of BASANT and the selected *Lactobacilli* strains to regress vaginosis in women. Besides testing the ability of these two individually, the efficacy of using combination of the two was also evaluated. Controls were taken, with Placebo capsules to assess the normal regression of the symptoms without treatment. This communication reports the findings.

MATERIALS

BASANT is a Polyherbal microbicide containing 95% purified diferuloyl methane {(E, E)-1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 -heptadiene-3, 5-dione} (Curcumin) from Curcuma longa, purified extract of Amla (Emblica officinalis), purified extract of Neem (Azadirachta indica) leaves, and Aloevera (Aloe barbadensis) extract. These ingredients are formulated in pharmacopoeially approved excipients: Citric Acid, Sorbitol, Microcrystalline Cellulose, Sodium Starchglycolate, Starlac, Crospovidones and Sodium alginate as a lubricating agent. 250 mg BASANT was dispensed as powder in cellulose capsules.

PROBIOTICS, *L. fermentum* (TRF#36), *L. gasseri* (TRF#8), and *L. salivarius* (TRF#30) were grown on a large scale by M/S Microbax, Hyderabad and made available in lyophilized form packed in cellulose capsules along with Prebiotics. The capsules utilized in the trial contained 3×10^9 of triple combination of *L. fermentum* (TRF#36), *L. gasseri* (TRF#8) & *L. salivarius* (TRF#30) (here after termed as Pro-veg-Health). These strains of Lactobacilli have been deposited in the National Depository at Institute of Microbial Technology Chandigarh, who have given them the following numbers: *Lactobacillus gasseri* (TRF #8) MTCC 5615, *Lactobacillus salivarius* (TRF#30) MTCC 5616, *Lactobacillus fermentum* (TRF#36) MTCC 5617.

MRS (deMan, Rogosa, and Sharpe) Broth and Agar (Hi-Media, India) were used for culture with Bromo-cresol purple (BCP, Merck, India), a colour indicator dye with pH range of 5.2- 6.8, the colour turns from purple to yellow with lowering of pH, enabling ready recognition of lactobacilli colonies.

The pH indicator strips were from EMD Chemicals, Gibbstown N.J. (USA), Associate of Merck KGaA, Darmstadt, Germany. The strips utilized for evaluating vaginal pH had pH range of 2.0 to 9.0. The pH above 5 and below 5 could be clearly visualized by colour change in one of the three rows

METHODS

Bacterial vaginosis was diagnosed clinically and by Amsel's criteria[10] for the presence of clue cells. Wet mount was prepared by directly taking discharge on a slide and adding one drop of 10 percent KOH to detect fishy odour (Whiff Test).

Gram staining & Catalase test

Lactobacillus bacteria are Gram positive and catalase negative. Gram staining was done by the standard method. Catalase test was done by pouring a drop of H_2O_2 on a colony. Absence of oxygen bubble formation indicated absence of catalase. Gram positive, catalase negative

colonies were cultured individually in MRS broth and stored in 20% glycerol at -20^{0}C.

Isolation of *Lactobacilli* from vaginal swab

Vaginal swabs in sterile tubes containing MRS Broth were brought to laboratory from the clinics. The sample was incubated at 37^{o}C overnight. The swab was spread on BCP-MRS agar plate and incubated at 37°; the colonies producing lactic acid were identifiable by the yellow colour.

DNA extraction[11]

DNA was extracted as per *Pospiech el al*[11].

Genus and Species Identification[12]

Genus and Species identification as per *Song et al*[12]. Each isolate was identified to the genus level by Polymerase Chain Reaction (PCR) with genus specific primer LbLMA-rev (5' CTC AAA ACT AAA CAA AGT TTC 3') and a universal primer R16-1 (5' CTT GTA CAC ACC GCC CGT TCA 3').

Species level identification was done with species specific primers of *L. fermentum, L. gasseri,* and *L. salivarius*. PCR program and reaction mixture was the same as for PCR-genus except annealing temperature which was 65^{o}C for *L. gasseri* (360 bp amplicon), and 60^{o}C for *L. fermentum* (192bp amplicon), and *L. salivarius* (411 bp amplicon). Following species specific primers were used:

L. fermentum

Lfer-3 : 5'ACTAACTTGACTGATCTACGA3'

Lfer-4 : 5' TTCACTGCTCAAGTAATCATC 3'

L. salivarius

Lsal-1 : 5' AATCGCTAAACTCATAACCT 3'

Lsal-2 : 5' CACTCTCTTTGGCTAATCTT 3'

L. gasseri

Lgas-3 : 5' AGCGACCGAGAAGAGAGAGA 3'

Lgas-2 : 5' TGCTATCGCTTCAAGTGCTT 3'

Amplicons were analysed by electrophoresis in 2% agars gel followed by ethidium bromide staining.

SUBJECTS & PROTOCOL

The criteria for enrolment of subjects were: Women between 18-45 years of age having regular cycles, clinical history of chronic and/ or recurring episodes of Bacterial vaginosis, willing to participate in the study after having been explained about the nature of the study and willing to return for follow up after 10 days of insertion of capsules and thereafter on monthly basis for 2 months or more. Women connectable at home by phone were given preference for enrolment in the trial, which enabled crosscheck of side effects if any after insertion of the capsules. The Exclusion Criteria were: known cases of diabetes mellitus and other systemic diseases including immuno-compromised states, suspected/confirmed pregnancy, suspected or known malignancy of reproductive tract, dysfunctional uterine bleeding, vaginal polyp and those less than 6 weeks since last delivery or abortion. Also excluded were those with severe allergic reactions and abnormality of vaginal anatomy likely to interfere with placement of drug, adnexal mass or tenderness and those having received a course of antibiotic therapy, less than 14 days prior to enrolment.

Eighty women were enrolled with history of repeated episodes of vaginosis, based on clinical symptoms like, thin, white, homogeneous discharge, Clue cells on microscopy of wet mount, pH of vaginal fluid >5.0 and release of fishy odour on adding alkali (10% KOH).The subjects were serially assigned to one of the four groups.

Group I was given 7 capsules of triple combination of Probiotics to be used one capsules of 3x10^9 *Lactobacilli* every night for a week. Group II was given 7 capsules of BASANT,one capsule to be taken every night for a week, Group III received combination of 7 capsules of BASANT and 7 capsules of Probiotics (combination hereafter termed as NAUROZ) to be used every night for a week and Group IV were controls who received 7 capsules of Placebo, one capsule every night for a week.On day 3, their experience of intravaginal insertion of the given capsules was asked by telephone and also, whether any side effects experienced. They were told to report to the clinic on day 10, when reexamination was done on the status of the discharge & other symptoms of vaginosis.Also recorded was the degree of relief experienced by the woman, pH was recorded and swab sample taken for culture.They were then given one capsule of the same to be taken once every week as maintenance regime. Their status was observed on repeat visits to the clinic or by telephone for atleast two months.

RESULTS

In group I women using Probiotics, 13 out of 20 (65%) were classifiable in effective category whereas 7 out of 20 did not respond to this treatment. Table 1 is an illustrative example of observations on a subject responding effectively to treatment with Probiotics and Table 2 represents a case in which treatment with Probiotics alone was not effective. While all 80 women enrolled in the trial reported to the clinic on day 10, thereafter those benefited continued to come at periodic intervals for receiving maintenance dose capsules to be taken once every week, Women who were not benefited ceased to come after some time and could be considered to be lost to follow up. All women enrolled however took for 7 days the prescribed capsules, reported on day 10 to the clinic for examination & thereafter received 2 maintenance capsules, one to be taken every week for the following two weeks.

Table 1: SUBJECT BV-A-95:- FALLING IN "EFFECTIVE" CATEGORY OF GROUP I GIVEN 7 CAPSULES OF PRO-VAG-HEALTH ONE CAPSULE EVERY NIGHT FOR 7 DAYS.

Patient ID BV-A-95	**Initial On Enrollment**	**1st Follow Up (Day 10)**	**2nd Follow Up (Day 31)**	**3rd Follow Up (Day 66)**	**4th Follow Up (Day 83)**
Date	22/03/12	02/04/12	23/04/12	28/05/12	14/06/12
Discharge Amount	+++	+	_	_	_
pH	5.0	4.5	5.0	4.5	4.5
Odour	+	_	_	_	_
Itching/burning	_	_	_	_	_
Clue Cells	+	_	_	_	_
Lactobacilli growth	_	+	+	+	+
Prescription	Pro-vag-Health (7 capsules)	Pro-vag-Health (2 capsules) one each week	Pro-vag-Health (2 capsules) One each week	Pro-vag-Health (2 capsules) One each week	Pro-vag-Health (2 capsules) One each week
Subjective Relief	_	95% Relief	100% Relief	100% Relief	Patient Satisfied
Side effects		None	None	None	None

"+" Present, "-"Absent

Table 2: SUBJECT BV-A-111:- FALLING IN " NOT-EFFECTIVE" CATEGORY OF GROUP I GIVEN 7 CAPSULES OF PRO-VAG-HEALTH ONE CAPSULE EVERY NIGHT FOR 7 DAYS.

Patient ID BV-A-110	**Initial On Enrollment**	**1st Follow Up (Day 14)**	**2nd Follow Up (Day 31)**
Date	09/04/12	23/04/12	10/05/12
Discharge Amount	+++	++	+++
pH	6.5	5.0	5.5
Odour	+	_	+
Clue Cells	+	_	+
Itching/burning	_	_	+
Lactobacilli growth	_	+	+
Prescription	Pro-vag-Health (7 capsules)	Pro-vag-Health (2 capsules) One each week	Pro-vag-Health (2 capsules) One each week
Subjective Relief	_	20% Relief	No Relief
Side effects		None	None

"+" Present, "-"Absent

In group II where women were using BASANT, 14 out of 20 women responded positively with healing of the symptoms. In group III in which women were treated

simultaneously with both BASANT and Pro-vag-Health (NAUROZ), surprisingly nearly every woman except one had a positive response. In group IV, in which Placebo Capsules were used by the 20 women for 7 days, only one woman showed regression of symptoms. Fig. 1 gives the four criteria on basis of which efficacy of the treatment were gauged. Table 3 is a summarized version of the observations in the 20 women enrolled in the NAUROZ group. Table 4 is the summary of the observations in the four groups of 80 patients receiving various treatments.

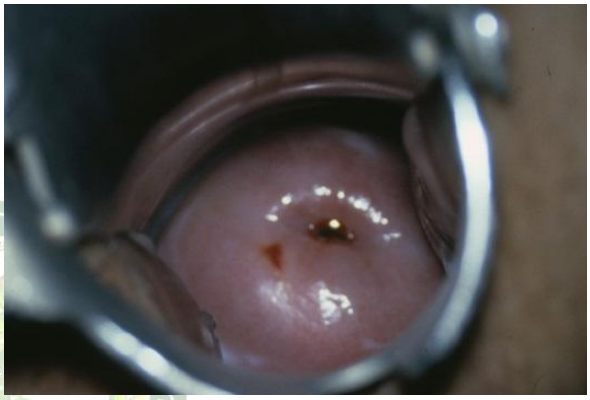
Statistical analysis was done to compare the proportion of patients in which regression of vaginosis occurred by different treatments as compared to control therapy. Analysis was carried out by Fisher's exact test/ Chi square test. All 3 therapies caused statistically significant improvement ($P<0.001$) as compared to control.

The Combination however was most effective (Table 4).

Fig 1: An illustrative representation of typical woman receiving the treatment with combination of BASANT plus Pro-vag-Health.

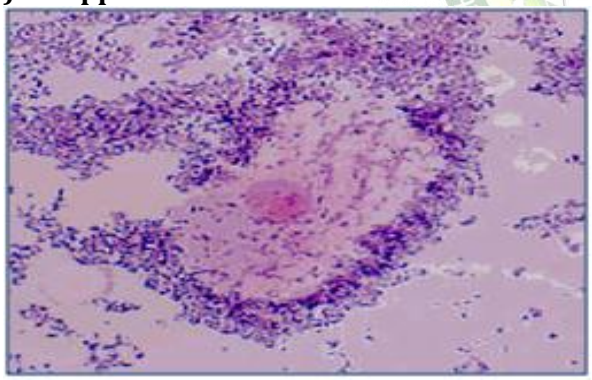
(a) Relief from abnormal vaginal discharge

On Enrollment

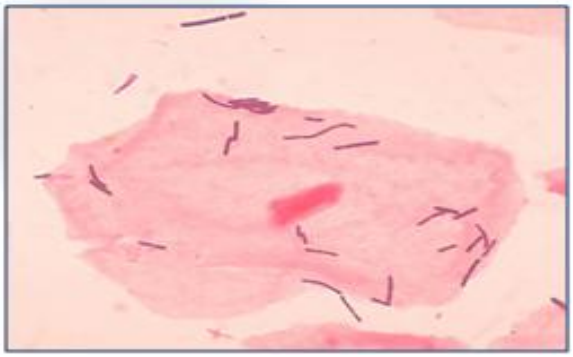


After Treatment

(b) Disappearance of Clue cells:



On Enrollment



After Treatment (Lactobacilli colonised)

(c) Healing of fishy odour:

Fishy Odour ⟶ **No Fishy Odour**

(After KOH addition on slide) (After KOH addition on slide)

On Enrollment **After treatment**

(d) pH of vagina restored to acidic range:

pH > 5 ⟶ pH < 4.5

On Enrollment **After treatment**

Table 3: EFFECT OF TREATMENT WITH COMBINATION OF BASANT PLUS PRO-VAG-HEALTH PROBIOTICS

Sl. No.	Pt. Id	VAGINAL DISCHARGE				SYMPTOMS (Itching/Burning)				ODOUR				CLUE CELLS				pH				LACTOBACILLI ISOLATED				SUBJECT RELIEF
		ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	
3.	BV-A-109	+++ +	+	_	_	++	+	_	_	+++	+	_	_	+++	+	_	_	**6.0**	**5.5**	**4.5**	**4.5**	_	+	+	+	**Patient Satisfied**
7.	BV-A-112	+++	+	_	_	++	_	_	_	+++	_	_	_	++	_	_	_	5.5	5.0	4.5	4.5	_	+	+	+	**Patient Satisfied**
11.	**BV-A-114**	**+++**	**++**	**++**	**++**	**+**	**+**	**+**	**+**	**+++**	**++ +**	**++**	**+**	**+++**	**++**	**++**	**++**	**5.0**	**5.0**	**5.0**		**_**	**_**	**_**	**_**	**Patient not Satisfied**
15.	BV-A-121	+++	+	_	_	++	_	_	_	++	_	_	_	++	_	_	_	5.0	4.5	4.5	4.0	_	+	+	+	**Patient Satisfied**
19.	BV-A-122	+++	+	_	_	++	_	_	_	++	+	_	_	+++	+	_	_	6.0	4.5	4.5	4.5	_	+	+	+	**Patient Satisfied**
23.	BV-A-123	+++	+	_	_	+	_	_	_	+++	_	_	_	++	_	_	_	5.5	5.5	4.5	4.5	_	+	+	+	**Patient Satisfied**
27.	BV-A-127	+++	_	_	_	+	_	_	_	+++	_	_	_	++	_	_	_	5.5	4.5	4.5	4.5	_	+	+	+	**Patient Satisfied**
31.	BV-A-128	+++	+	_	_	++	_	_	_	++	_	_	_	+++	_	_	_	5.5	5.0	4.5	4.0	_	+	+	+	**Patient Satisfied**
35.	BV-A-132	+++ +	+	_	_	++ +	_	_	_	+++	_	_	_	++	_	_	_	5.5	4.5	4.5	4.5	_	+	+	+	**Patient Satisfied**
39.	BV-A-134	+++ +	+	_	_	++	_	_	_	++	_	_	_	+++	_	_	_	5.5	4.5	4.5	4.5	_	+	+	+	**Patient Satisfied**

"+" PRESENT "-" NOT PRESENT "ENR" ENROLLMENT

Table 3: EFFECT OF TREATMENT WITH COMBINATION OF BASANT PLUS PRO-VAG-HEALTH PROBIOTICS

Sl. No.	Pt. Id	**VAGINAL DISCHARGE**				**SYMPTOMS (Itching/burning)**				**ODOUR**				**CLUE CELLS**				**pH**				**LACTOBACILLI ISOLATED**				**SUBJECT RELIEF**
		ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	ENR	Day 10	Day 25	Day 60	
43.	BV-189	**+++ +**	**+**	**–**	**–**	**++**	**–**	**–**	**–**	**+++**	**–**	**–**	**–**	**+++**	**–**	**–**	**–**	**8.5**	**5.0**	**4.5**	**4.5**	**–**	**+**	**+**	**+**	**Patient Satisfied**
47.	BV-190	+++	+	–	–	++	–	–	–	++	–	–	–	++	–	–	–	8.0	5.0	4.5	4.5	–	+	+	+	**Patient Satisfied**
51.	BV-191	+++	–	–	–	++ +	–	–	–	+++	–	–	–	++	–	–	–	7.0	5.0	4.5	4.0	–	+	+	+	**Patient Satisfied**
55.	BV-195	+++	+	–	–	+	–	–	–	++	–	–	–	+++	–	–	–	7.5	4.5	4.5	4.0	–	+	+	+	**Patient Satisfied**
59.	BV-196	+++	+	–	–	++	–	–	–	++	–	–	–	++	–	–	–	7.5	5.0	4.5	4.5	–	+	+	+	**Patient Satisfied**
63.	BV-197	+++	+	–	–	++	–	–	–	+++	–	–	–	+++	–	–	–	8.5	5.0	4.5	4.5	–	+	+	+	**Patient Satisfied**
67.	BV-198	+++	–	–	–	++	–	–	–	++	–	–	–	++	–	–	–	7.5	5.0	4.5	4.5	–	+	+	+	**Patient Satisfied**
71.	BV-200	++	–	–	–	+	–	–	–	++	–	–	–	++	–	–	–	8.5	4.5	4.5	4.5	–	+	+	+	**Patient Satisfied**
75.	BV-201	++	–	–	–	+	–	–	–	++	–	–	–	++	–	–	–	6.5	5.0	4.5	4.5	–	+	+	+	**Patient Satisfied**
79.	BV-203	+++	+	–	–	++	–	–	–	++	–	–	–	++	–	–	–	7.0	6.0	4.5	4.5	–	+	+	+	**Patient Satisfied**

"+" PRESENT "-" NOT PRESENT "ENR" ENROLLMENT

Table 4: SUMMARY OF RESULTS OF TREATMENT WITH EITHER PROBIOTICS, BASANT OR A COMBINATION OF BASANT AND PROBIOTICS TO CURE VAGINOSIS AND RESTORE REPRODUCTIVE HEALTH

Group Name	Improved (%) N=20	P value Comparison with Placebo	P value Comparison with BASANT+Probiotics
Probiotics	65%	P<0.001	P = 0.04
BASANT	70%	P<0.001	P = 0.09
BASANT + Probiotics	95%	P<0.001	-
Placebo	5%	-	P<0.001

Comparison with Fisher's exact test

DISCUSSION

As pointed out in Introduction, vaginosis is a widely prevalent syndrome, 30% to 80% women in urban and rural areas are suffering from vaginosis. For cultural reasons they do not normally complain and consider it as a part or consequence of marriage and intercourse. This study was carried out in two major hospitals of Delhi where women coming for antenatal or fertility related problems were diagnosed to suffer from vaginosis. Normally these women are given treatment with antibiotics like Metronidazaole, Clindamycin plus Clotrimazole which in most cases give relief, even though it is not long lasting and recurrence of vaginosis is frequent. This problem is not only restricted to India but is also noted to occur in other countries.

Treatment with antibiotics does not always restore the in-dwelling Probiotic lactobacilli in the vagina with the result that the vaginal pH of such patients is frequently above 5.0 and they remain prone to contracting infections again. One of the objectives of the present trial was to ascertain whether the three strains of *Lactobacilli* previously isolated from the healthy vagina and short listed from amongst 80 strains on the basis of their high production of lactic acid, hydrophobicity and arginine deiminase activity do colonise in vagina. Another objective of the trial was to see whether a Polyherbal microbicide BASANT endowed with wide spectrum antimicrobial activity can replace the antibiotics currently prescribed. BASANT is competent to regress vaginosis though only in 14 out of 20 (70%) of women. BASANT capsules were well received with ease of insertion and lack of any side effects or adverse events. Thus the Polyherbal microbicide composed of purified quality controlled ingredients from traditionally used herbs, formulated as powder delivered in cellulose capsules, has the potential of use as an OTC product. Besides acting against the spectrum of aerobic and anaerobic microorganisms causing vaginosis, BASANT is endowed with pronounced inhibitory action against a number of sexually transmitted infections like *Staphylococcus aureus, Neisseria gonohrreae, Chlamydia trachomatis, Candida spp,* HPV and HIV. In an *ongoing trial* in women at early stage of Cervical dysplasia, when the causative HPV is not yet integrated in the genome, 30 applications of BASANT capsule, one each night, eliminated HPV and restored the cervical cytology and Pap smear to normal type.

Although each one of the three selected Probiotic strains namely TRF#36 strain of *Lactobacillus fermentum*, TRF#8 strain of *L. gasseri*, and TRF#30 strain of *L. salivarius* is able to colonise in the vagina, administration of the combination of three (Pro-veg-Health) colonised in almost all women who received the triple combination. It took care of the person to person variability and sexual behaviour amongst women.

Even though Probiotics or BASANT gave relief individually and were useful in many but not all, the Combination of the two NAUROZ was highly effective, 19 out of 20 taking a combination of BASANT and Pro-vag-Health Probiotics (NAUROZ) for seven days were fully cured in terms of the regression of vaginal discharge, pH returning to acidic range, absence of clue cells and *lactobacilli* colonised in the vagina.

CONCLUSION

These studies indicate the merit of using combination of a wide spectrum microbicide with Probiotics of meritorious properties, for regression of vaginosis & restoration of vaginal health. The combination has high efficacy of

curing vaginosis, bringing down the vaginal pH to acidic range accompanied by colonisation of Probiotic *Lactobacilli.*

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