



Clinical Research: Gynaecology and Obstetrics

Research Article

Clinical Research Leading to Development of Two Products for Keeping Vagina Healthy and Preventing Sexually Transmitted Infections

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Abstract

Described below are 2 Products developed by us which after undergoing successful clinical trials and approval of the Drugs Controller General of India (DCGI) and Institutional Ethics Committees, have been transferred to Industry for making these available to potential users. These are: [1] a Polyherbal formulation BASANT with inhibitory action on a wide spectrum of genital pathogens, and [2] 3 strains of Lactobacilli (Pro-vag-Health) that make and secrete high amounts of lactic acid, are hydrophobic to enable colonization and possess arginine deiminase, the enzyme preventing the formation of foul odour derivatives. The combined use of BASANT and pro-vag-Health in the Product NAUROZ, has the highest efficacy ever achieved for curing recurrent episodes of vaginosis.

Introduction

Vagina is a unique organ. It is the portal which makes possible reproduction. Besides sperms, it is open to invasion by aerobic and anerobic micro-organisms including pathogens causing a variety of sexually transmitted infections such as gonnorrhoea, HIV, etc. While a variety of

methods are available to women for contraception, there is need for good

Microbicides which may be easy and safe to use and have the ability to prevent a wide spectrum of infections. Described below is a unique microbicide developed by us.

Polyherbal Microbicide BASANT

BASANT is composed of diferuloyl methane (curcumin), purified extracts of *Emblca officinalis* (Amla), *Aloe barbadensis* (Aloe vera), and *Azadirachta indica* (Neem) leaves along with pharmacopoeially approved excipients and preservatives. It is packaged in cellulose capsules which are easily insertable by women in vagina.

BASANT inhibits the growth of all WHO (World Health Organization) strains of *Neisseria gonorrhoeae*, including those resistant to Penicillin, Tetracycline, Nalidixic acid and Ciprofloxacin (Table 1).

Table (1): WHO strains of *Neisseria gonorrhoeae* inhibited by BASANT (adapted from 1)

Standard WHO strains	Antibiotic resistance	Growth at different concentrations (v/v) of Basant after 24 h			
		5%	2%	1%	0%
<i>N. gonorrhoeae</i> WHO-C	None	-	-	+	+
<i>N. gonorrhoeae</i> WHO-G	Tetracycline (TRNG), nalidixic acid	-	-	-	+

<i>N. gonorrhoeae</i> WHO-K	Penicillin (PPNG), nalidixic acid, ciprofloxacin, ceftriaxone less sensitive	-	-	-	+
<i>N. gonorrhoeae</i> WHO-L	Penicillin (PPNG), nalidixic acid, ciprofloxacin, ceftriaxone less sensitive	-	-	-	+
<i>N. gonorrhoeae</i> 1586	Nalidixic acid, ciprofloxacin	-	-	-	+
<i>N. gonorrhoeae</i> 1669	Nalidixic acid, ciprofloxacin	-	-	+	+
<i>N. gonorrhoeae</i> 1794	Nalidixic acid, ciprofloxacin	-	-	+	+
<i>N. gonorrhoeae</i> 2182	Nalidixic acid, ciprofloxacin	-	-	-	+
<i>N. gonorrhoeae</i> 2436	Penicillin (PPNG), tetracycline (TRNG), nalidixic acid,	-	-	-	+

	ciprofloxacin				
<i>N. gonorrhoea</i> e 2482	Penicillin (PPNG), nalidixic acid, ciprofloxacin	-	-	-	+
<i>N. gonorrhoea</i> e 2676	Penicillin (PPNG), tetracycline (TRNG), nalidixic acid, ciprofloxacin less sensitive	-	-	+	+

It has pronounced inhibitory action against *Candida glabrata*, *Candida albicans* and *Candida tropicalis* isolated from women with vulvo-vaginal candidiasis, including 3 strains resistant to azole drugs and amphotericin B Table (2).

Anti-HIV action of BASANT

The ability of BASANT to inhibit HIV has been documented by a number of determinations. At 1:20,000 dilution, it causes 50% inhibition, whereas at 1:1000 dilution, it inhibits nearly totally the replication of the virus in 2 types of cells as hosts. Gustavo Doncel at CONRAD in USA who carried out these investigations observed also that Aloe Vera had the highest inhibitory activity against HIV amongst the components of BASANT [1].

Table (2): Clinical isolates of *Candida* species inhibited by BASANT (adapted from 1)

Clinical isolate of <i>Candida</i> Species	Antibiotic resistance	Growth at different concentrations (v/v) of Basant after 24 h			
		5 %	2 %	1%	0 %
<i>C. glabrata</i> ATCC 90030	None	0	0	0-0.064 × 10 ⁸ CFU/mL	1.38-1.49 × 10 ⁸ CFU/mL
<i>C. glabrata</i> (7 clinical isolates)	None	0	0	0-0.090 × 10 ⁸ CFU/mL	1.30-1.50 × 10 ⁸ CFU/mL
<i>C. glabrata</i> (2 clinical isolates)	Fluconazole, itraconazole, ketoconazole, voriconazole, amphotericin B	0	0	0-0.075 × 10 ⁸ CFU/mL	1.40-1.50 × 10 ⁸ CFU/mL
<i>C. albicans</i> ATCC 36082	None	0	0	0-0.077 × 10 ⁸ CFU/mL	1.40-1.47 × 10 ⁸ CFU/mL

<i>C. albicans</i> (5 clinical isolates)	None	0	0	0.05-0.08 × 10 ⁸ CFU/mL	1.37-1.50 × 10 ⁸ CFU/mL
<i>C. albicans</i>	Fluconazole, itraconazole, ketoconazole, voriconazole, amphotericin B	0	0	0-0.067 × 10 ⁸ CFU/mL	1.33-1.40 × 10 ⁸ CFU/mL
<i>C. tropicalis</i> (2 clinical isolates)	None	0	0	0.04-0.07 × 10 ⁸ CFU/mL	1.35-1.41 × 10 ⁸ CFU/mL

Prof. Manoj Pастey at the University of Oregon, USA has reported that BASANT exercises inhibitory action against both CCR5 and CXCR4 tropic HIV strains [2]. Dr. Smita Kulkarni at the National Institute of Virology Pune has found that BASANT inhibits HIV-1 replication in Epi vaginal explants model.

Table (3): Percent inhibition of virus production by Basant in HIV-1 NL4.3-infected CEM-GFP and P4 cells (adapted from 1).

Basant Dilution	% Inhibition by p24 assay in	
	CEM-GFP cells	P4 cells
1:1000	98	99
1:5000	89	Not calculated
1:10000	81	64
1:20000	51	53

1:40000	82	45
1:80000	Not calculated	32

Anti-HPV16 action of BASANT

Human Papilloma virus 16 and 18 are amongst those strains of HPV, which are responsible for carcinoma of cervix. John Schiller at the National Cancer Institute of National Institute of Health Bethesda in USA, observed that the 2 ingredients of BASANT namely Aloe vera (Aloe barbadensis) and Amla (Embllica officinalis) inhibited the transduction of HPV-16 in Hela-cells at concentrations far below those that are cytotoxic and those used in the formulation (Table 4).

Table 4. Inhibition of HPV-16 infectivity by BASANT and its ingredients (adapted from 1).

Compound	Cytotoxicity (µg/mL) ^a	Infectivity inhibition (EC ₅₀) (µg/mL)
Basant	300 dilution ^b	~ 2700 dilution ^c
Aloe	> 100 dilution	1800 dilution
Amla	300	4
Curcumin	5	>5
Saponins	100	>100

EC₅₀, 50% effective concentration
^aCytotoxicity is defined as a >50% decrease in WST-1 substrate (Roche) development
^bFold dilution
^cInhibition curve could not be fitted

An extremely useful property of BASANT is not only to restrict the entry of HPV-16 into

cervical cells, but also to eliminate HPV-16 from the virus infected cervical cells on way to progression towards carcinoma of cervix. A trial was conducted at the Jawaharlal Nehru Medical College, Aligarh in collaboration with the Institute of Cytology and Preventive Oncology, of Indian Council of Medical Research. One hundred fifty nine women coming to Obstetrics and Gynaecology clinic were examined clinically by visual inspection of cervix and by staining with acetic acid. Cervical scraps were collected from the ecto-cervical region and endo-cervical canal to prepare Pap smears. Thirty five women were enrolled who had inflammatory cervix and abnormal Pap smear. Out of these, 19 women were found positive for HPV-16. Eleven women out of these 19 gave written consent to undergo intra-vaginal treatment with BASANT. They were asked to insert a capsule containing 245 mg of BASANT in the vagina every night before going to bed for 30 days, excluding the days of menstruation. Table 5 summarises the observations.

Table 5. Pre and post treatment with BASANT of HPV-16 positive patients (adapted from 3)

S.No	Age	Parity	HPV-16 Pre-treatment	HPV-16 Post-treatment
1	42	3+0	+VE	NEGATIVE
2	27	4+0	+VE	NEGATIVE
3	35	3+0	+VE	NEGATIVE
4	28	1+1	+VE	NEGATIVE
5	45	3+0	+VE	NEGATIVE
6	35	4+0	+VE	NEGATIVE

7	30	2+1	+VE	NEGATIVE
8	45	2+0	+VE	NEGATIVE
9	38	5+2	+VE	NEGATIVE
10	35	3+1	+VE	NEGATIVE
11	38	3+1	+VE	NEGATIVE
Number of patients screened for HPV DNA: 159; Number of patients positive for HPV-16: 19; Intravaginal BASANT given with written consent to 11 HPV positive patients accepting to take BASANT; All turning HPV-16 negative after using intravaginally BASANT.				

What was amazing was that every woman (11/11) who used BASANT for 30 nights, had no HPV-16 in their cervical cells. Pap smear, which was abnormal to begin with, became normal in each case [3]. Thus BASANT can be extremely helpful to women to get rid of infection of HPV-16 in their cervical cells at early stages of CIN I, II, so long as the virus is not integrated in the host genome. The only disadvantage of using BASANT is the yellow coloration that it imparts to vaginal fluids during usage, which can easily be taken care of by employing vaginal pads.

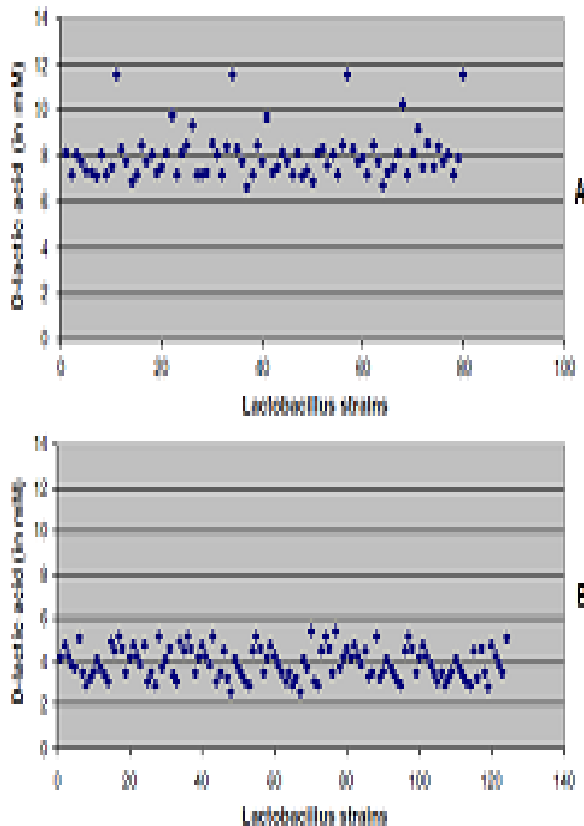
Probiotics

Lactobacilli are friendly microbes that co-exist with cells of the vagina. They make and secrete Lactic acid locally bringing down the pH of healthy vagina to 4-4.5. The acidic pH of healthy vagina is in contrast to pH 7.4 of the body. The acidic pH restricts locally several micro-organisms to infect the vagina. Many strains of lactobacilli, also make and secrete H₂O₂. Good strains of lactobacilli have the enzyme arginine deiminase, which

prevents the production of foul odour derivatives.

Several species of Lactobacilli inhabit vagina and each species has several strains differing in their metabolic properties. There are strains that make large amount of lactic acid and there are strains of even the same species that are low secretors [4]. Figure (1) shows the disparity in the amount of lactic acid made by different strains of Lactobacillus.

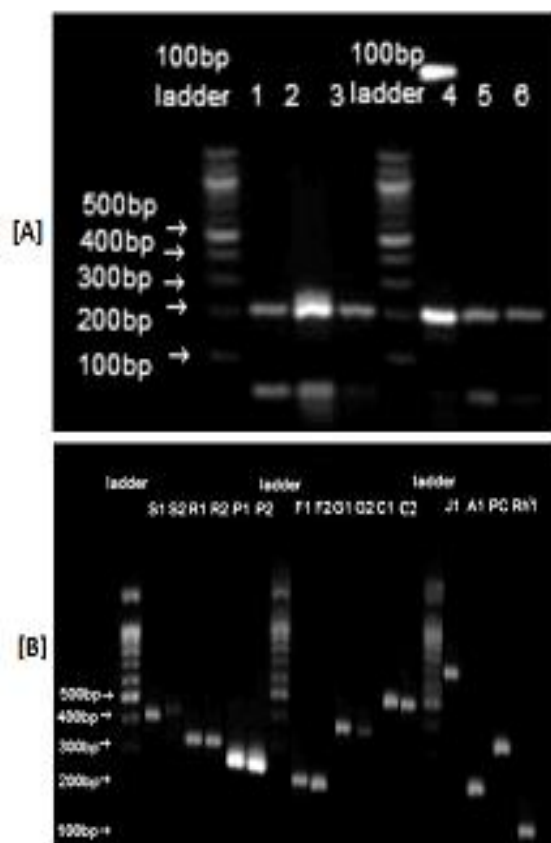
Figure (1): (A) D-lactic acid production by 80 Lactobacillus strains from women with healthy vagina (B) D-lactic acid production by 124 Lactobacillus strains from women with Bacterial Vaginosis (BV) (adapted from 4)



On basis of their ability to make and secrete high amounts of lactic acid, their high hydrophobicity which enables colonization in the vagina and positivity of containing

arginine deiminase, we selected 3 meritorius strains of Lactobacilli referred to as Pro-vag-Health probiotics. These are *L. salivarius* TRF # 30, *L. fermentum* TRF # 36, and *L. gasseri* TRF # 8. These have been patented and deposited with the International Collection Depository. Figure (2) shows their identification and characterization by genus specific Polymerase Chain Reaction (PCR) and species specific PCR.

Figure (2): [A] Representative genus specific PCR products of 6 isolates (lanes 1-3, 4-6) on agarose gel electrophoresis. All isolates gave a 200 bp amplicon. [B] Species specific PCR products of the 10 species of Lactobacilli isolated from healthy vagina. This representative figure shows the profiles of 2 isolates of each of the 6 species and 1 isolate of the rest of 4 species as viewed on 2% agarose gel. S1 and S2, *L. salivarius* strains, 400 bp amplicon; R1 and R2, *L. reuteri* strains, 300 bp amplicon, P1 and P2, *L. plantarum* strains, 250 bp amplicon; F1 and F2, *L. fermentum* strains, 200 bp amplicon; G1 and G2, *L. gasseri* strains, 350bp product; C1 and C2, *L. crispatus* strains, 500 bp PCR amplicon; J1, *L. jensenii*, 700 bp amplicon; A1, *L. acidophilus*, 200 bp PCR amplicon; PC, *L. paracasei*, 300 bp amplicon; Rh1 *L. rhamnosus*, 100 bp amplicon (adapted from 5).



High efficacy of BASANT + Pro-Vag-Health Probiotics for Curing Vaginosis

Vaginosis is prevalent in about 30% of women in urban localities [6] and in about 50% of women in rural areas [7]. Even in USA, 29% of women suffer from it [8]. Vaginosis is manifested as abnormal vaginal discharge accompanied by Clue cells, and pH of vagina above 5. It is caused by an array of aerobic and anerobic micro-organisms.

With approval of the Drugs Controller General of India and Institutional Ethics Committees, Phase II clinical trials were conducted at the All India Institute of Medical Sciences, New Delhi and Sir Gangaram

Hospital, New Delhi in 80 women suffering from recurring episodes of Vaginosis. 20 Women were given the Pro-vag-Health lot of 3 selected Probiotics and 20 women were treated with BASANT. 20 Women used both BASANT and probiotics, and 20 women were kept on placebo capsules. Table (6) gives the results.

While Probiotics and BASANT cured 65 to 70% of women, the combination of the 2 (NAUROZ), cured nearly every woman, 19 out of 20 (95% efficacy). This is the highest order of efficacy of any product available to treat vaginosis clinically in the world literature. Metronidazole and other antibiotics currently employed, attain only an efficacy of about 65%. Fig. 3 illustrates the efficacy of the combination in one of the 19 patients.

Table (6): Summary of results of treatment with either Probiotics, BASANT or a combination of BASANT and Probiotics to cure Vaginosis and restore reproductive health (adapted from 9)

Group Name	Women enrolled	Women Improved	P value Comparison with Placebo	P value Comparison with BASANT +Probiotics
Probiotics	20	13 (65%)	P<0.001	P = 0.04
BASANT	20	14 (70%)	P<0.001	P = 0.09
BASANT +Probiotics	20	19 (95%)	P<0.001	-

(NAUROZ)				
Placebo	20	1 (5%)	-	P<0.001
Comparison with Fisher's exact test				

These 3 selected strains of Lactobacilli (Pro-vag-Health) have been transferred to M/s Micobax Hyderabad. Vikas Rajurkar of M/s Microbax (India) Ltd can be contacted for these probiotic strains (Email: vikasrajurkar@microbax.com). BASANT has been licensed to M/s Bipha Drug Laboratory Pvt Ltd, Kerala. The Managing Director, Mr. Ajay George Varghese (Email: ajay@bipha.com) can be approached for the requirements of BASANT.

Figure (3): An illustrative representation of a typical woman receiving the treatment with NAUROZ, a combination of BASANT plus Pro-vag-Health (adapted from 9).

(a) Relief from abnormal vaginal discharge

On Enrollment

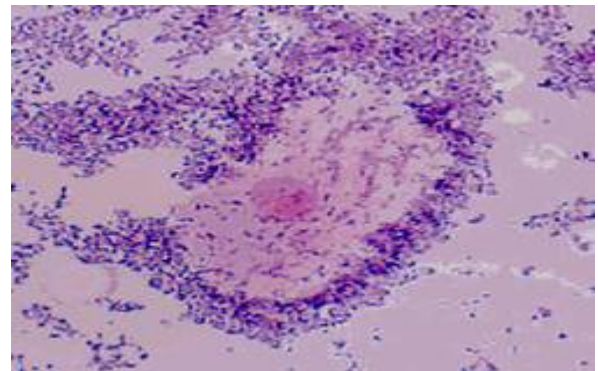


After Treatment

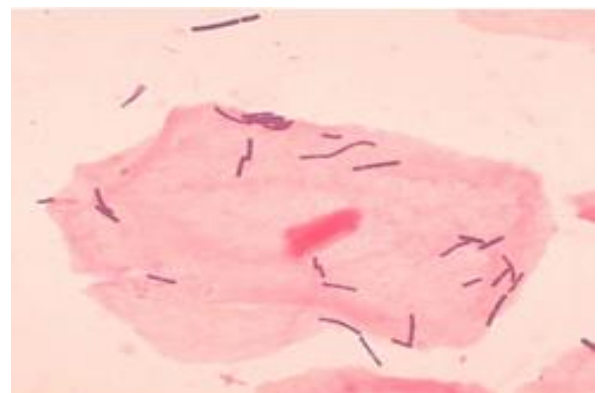


(b) Disappearance of Clue cells

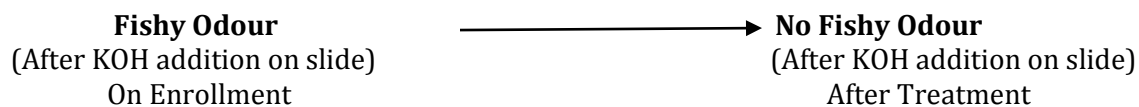
On Enrollment



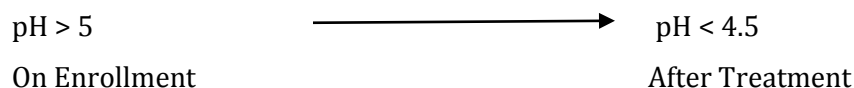
After Treatment (Lactobacilli colonised)



(c) Healing of fishy odour



(d) pH of vagina restored to acidic range



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