Original Article

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Clinical trial on safety and acceptability of a Polyherbal Vaginal Microbicide Cream: BASANT

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Abstract

A vaginal microbicide cream (Basant) formulated from diferuloylmethane (curcumin), extracts of Emblica officinalis, Sapindus mukorossi, Aloe vera and Rose water has anti-microbial, anti-human papilloma virus and anti-human immunodeficiency virus action in vitro. Pre-clinical toxicology on rabbits showed it to be safe. This study was conducted to evaluate its safety and acceptability in humans.

Methods: A total of 30 sexually active women used it for 14 days. Clinical examination, Pap smear, colposcopy, cervicovaginal lavage (CVL) for cytokines, vaginal swabs, urine and blood samples for sexually transmitted infections, hematology and biochemistry were collected at baseline and post-cream use. The paired 't test' was used for intra-group comparison of means, and Wilcoxon signed ranks test for cytokine data.

Results: The mean age was 31.5 years. Baseline findings were normal except vaginitis in 10/30 and inflammatory Pap smear in 11/30, they were treated before enrolement. Women with vaginitis were treated with a single oral dose of Secnidazole and Fluconazole and vaginal tablets (Clotrimazole + Tinidazole) for 6 nights. Those with inflammatory Pap were treated with vaginal tablets only. Post-cream use, examination was normal in 29/30; one had vaginal candidiasis. Hematology and blood biochemistry were similar to baseline except for mean aspartate aminotransferase and creatinine levels which were elevated significantly, though within normal range. In CVL, interleukin (IL)-6 and interferon -γ (IFN-γ) were elevated and IL-1-β, IL-2, IL-12 and tumor necrosis factor -α (TNF-α) were lowered significantly from baseline. Grade 2 toxicity (division of acquired immunodeficiency syndrome) was observed in 2/30; one had candida albicans in urine and one had hyperkalemia. Grade 1 toxicity was observed in 12/30, mainly transient vaginal irritation. Penile irritation was reported by 3/26 following intercourse. The women initial acceptability score was 10/10 (100%) and follow-up was 21.83 ± 1.66 (range 19-25/25) showing acceptability of 87.32%. In men, the follow-up score (among 26/30 sexually active couples) was 4.53 ± 0.91 (range 1-5/5), showing acceptability of 90.6%.

Conclusions: Basant cream was safe and acceptable. The toxicities were mild and transient. It did not increase IL-1- β in CVL like other irritating products, though elevated IL-6 and IFN -γ need to be observed in future to assess their impact on mucosal inflammation.

Key words: Cervicovaginal lavage, cytokines, microbicide, polyherbal, vaginal, vaginitis

Introduction

The World Health Organization (WHO) reported over a 333 million new cases of sexually transmitted infections (STIs) excluding human immunodeficiency virus (HIV) occurring annually world-wide, with the majority in developing countries [1]. In India, the prevalence of reproductive tract infections (RTIs) was reported as 41% in Delhi, 20.5% in rural Karnataka, 48.5% in Haryana and 50% in rural Maharashtra [2-6]. Microbicides may protect women from RTIs, several STIs and HIV. These are formulated for application into the vagina and/or rectum to prevent sexual transmission of organisms [7]. Microbicides are classified by their primary mechanism of action [8]. Vaginal defense enhancers maintain acidic pH and facilitate colonization with lactobacilli (acidform , buffergel, and probiotics). Surfactants disrupt microbial cell membranes (Nonoxynol-9 or N-9), but N-9 also disrupts genital epithelium and may enhance HIV acquisition [9]. Entry or fusion inhibitors target viral epitopes or cell receptors (CD4, CCR5, CXCR4) to prevent viral binding and entry into cells (PRO 2000, cellulose sulfate). Cellulose sulphate Phase III trials were stopped due to possible increase in HIV infection [10]. Viral replication inhibitors inhibit HIV-1 reverse transcriptase enzyme, i.e. Tenofovir. Pericoital use of 1% tenofovir gel in CAPRISA 004 study reduced HIV-1 acquisition by 39% and herpes simplex virus-2 acquisition

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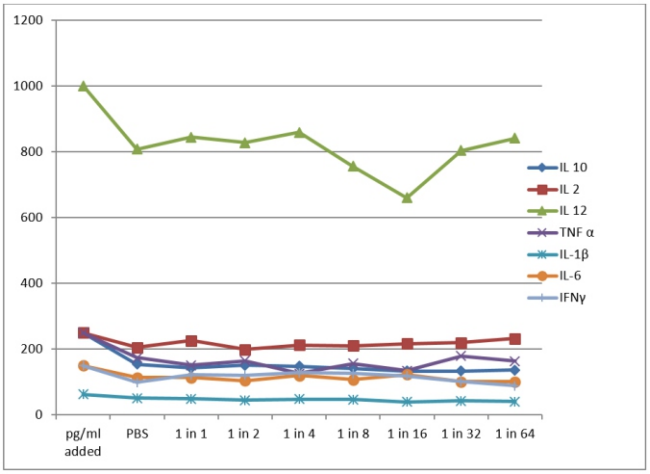

Figure 1: Basant cream interference with cytokine assay. Cytokine values obtained after PBS and Basant cream solution (3 ml cream in 10 ml PBS) at dilutions from 1:1 to 1:64 were spiked with known amount of each cytokine. The recovery was good, as seen by the nearly straight lines in this figure.

by 51% [11]. Two polyherbal microbicides with multiple modes of action have been develop in India and are Praneem and Basant. Praneem has extracts from Azadirachta indica, Sapindus mukerosi and Mentha citrata oil and was used to treat STIs [12]. Its in-vitro antiretroviral action led to its development as a potential candidate HIV microbicide [13-16]. However, vaginal irritation and an unpleasant odor did not make it popular. The constituents of Basant formulated as a cream and a powder in a vegetable (cellulose) capsules are purified diferuloyl methane ([E, E]-1,7- bis [4-hydroxy-3-methoxyphenyl] -1,6-heptadiene-3,5-dione) commonly known as curcumin (from turmeric or Curcuma longa), purified extract of Emblica Officinalis (Indian gooseberry or amla), purified saponins from Sapindus mukerossi (reetha), Aloe vera and rose water. These ingredients are formulated in pharmacoepially approved excipients; glycerol, PEG-400, alginic acid, xanthan gum, lactic acid, citric acid, potassium-sodium tartrate; and benzoic acid as a preservative. Its pH is 3.5. Its buffering activity was estimated by determining pH after adding phosphated buffer saline (PBS) of pH 8.0 to the cream in varying proportions (1:1 to 1:9). Even when 1 part cream was mixed with 9 parts PBS, the pH remained ~4.0. Thus, Basant cream can be expected to maintain an acidic vaginal pH despite the alkaline pH of semen. The inclusion of its ingredients is based primarily on their anti-microbial, anti-inflammatory, anti-human papilloma virus (HPV) and anti-HIV activity. Basant cream inhibits the growth of Neisseria gonorrheae, urinary tract Escherichia coli (including multi-drug resistant strains), Staphylococcus aureus, Candida albicans (including fluconazole resistant strains), Candida glabarata, Candida krusei, Candida parapsillosis and Candida tropicalis. It has high virucidal action on HIV, as determined by Dr Debashis Mitra at NCCS, Pune, India on 2 different cell lines and by Dr Gustavo F Doncel at CONRAD lab, Norfolk USA. Its ingredients prevent the transduction of HPV-16 as determined by Dr John Schiller at National Institute of Cancer, NIH, Bethesda [17]. Preclinical toxicology on rabbits by Dr KVR Reddy at the National Institute for Research in Reproductive Health, Mumbai, India showed it to be safe. The present study was carried out to evaluate the local and systemic safety and acceptability of Basant cream in reproductive age group sexually active HIV uninfected women and to see its effect on vaginal microflora and cytokine profile in cervicovaginal lavage (CVL).

Material & Mathod

The study was conducted between May 2009 to July 2010 in the Department of Obstetrics & Gynaecology of the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India after approval of the Institute's ethics committee. A total of 30 healthy women of age 18-45 years with regular menses who were sexually active with a single male partner, both HIV negative, were enrolled. Women who were pregnant or breast feeding or using an intrauterine device or had a genital lesion, or STIs were excluded. The women reported as follows:

Screening visit: The women were subjected to a patch test with the cream on the volar surface of the forearm to exclude those allergic to this cream. Clinical and pelvic examination were performed, vaginal pH noted and vaginal swabs collected for wet smear and Gram's stain for Trichomonas vaginalis, Candida vaginitis and bacterial vaginosis (BV) by Nugent's criteria. A Pap smear was collected. Blood sample from the couple were drawn for HIV, venereal disease research laboratory (VDRL), Hepatitis B antigen (Hbs) and from women alone for hemogram, coagulogram and biochemistry. Urine was collected for routine examination, culture and polymerase chain reaction (PCR) (for Chlamydia and Gonococcus). Women with vaginitis and/or an inflammatory Pap smear were treated prior to enrollment. For vaginitis, a single oral dose of Secnidazole 2 g plus Fluconazole 150 mg and vaginal tablets for 6 nights (Clotrimazole 200 mg + Tinidazole 500 mg) were given. Women with an inflammatory Pap smear were treated only with the above mentioned vaginal tablets. Enrollment visit (day 7-10 of next menstrual

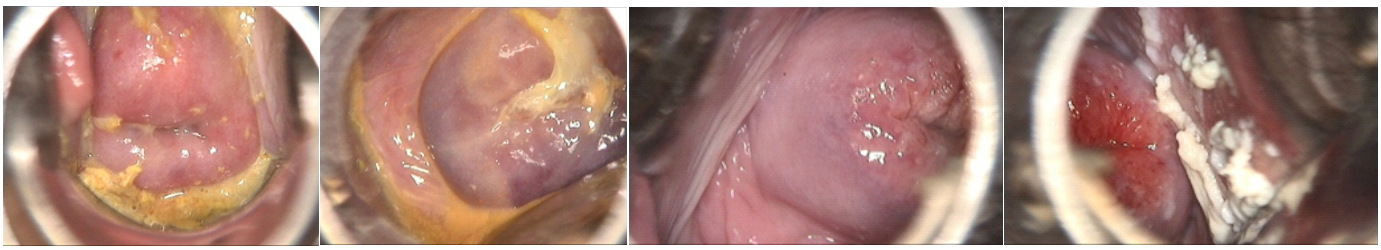

Figure 2: Upper row: Colposcopic pictures of two women: Code 026 and 012, one day (~12 hours) and 2 days (~36 hours) after last cream application. Lower row: Colposcopy A (baseline) and C (cervical erythema with vaginal candidiasis) in one woman (code 033).

Table 1: Baseline and post-cream use hematology and blood biochemistry indices

	Test/ Parameter	Normal lab range	Baseline (Mean ± SD)	Baseline range	Post cream use (Mean ± SD)	Post cream use range	paired t test	p value
1	Hb (G/dl)	43452	10.99 ± 1.09	9.20 -13.40	10.80 ± 1.019	9.20 – 12.4	1.859	0.073
2	TLC (/mm3)	4000- 11000	7123.3 ± 1382.6	4900 - 10900	6976.7 ± 1396.7	4000 - 10000	0.597	0.555
3	PCV (%)	36- 54	34.07 ± 3.53	27.10 – 39.2	32.46 ± 4.51	20 – 39.3	1.972	0.058
4	ESR (mm)	0-20	26.13 ± 10.28	4 – 46	26.29 ± 9.95	16954	0.068	0.946
5	Platelets (lac/mm3)	1.5 - 4.0	2.5 ± 0.73	1.47 - 3.77	2.55 ± 0.92	1.44 - 5.14	0.463	0.647
6	PT (sec)	43451	13.4 ± 0.93	12 – 17	13.36 ± 0.85	12 – 15	0.171	0.865
7	PTI (%)	80 - 100	93.44 ± 7.38	70 – 100	92.47 ± 6.43	80 - 100	0.716	0.48
8	INR	0.8 - 1.2	1.05 ± 0.07	0.95 - 1.3	1.05 ± 0.67	1- 1.2	0.646	0.523
9	B. sugar (mg/dl)	70 - 140	92.43 ± 14.06	55 – 129	98.07 ± 16.39	72 - 138	0.821	0.42
10	B. urea (mg/dl)	18537	22.82 ± 5.301	11.5 - 37.9	23.15 ± 5.22	15.7 - 38.3	1.605	0.122
11	S. creatinine (mg/dl)	0.50 - 1.2	0.82 ± 0.15	0.47 - 1.2	0.91 ± 0.185	0.53 - 1.31	2.387	0.024*
12	S. uric Acid (mg/dl)	3.4 – 7.0	4.05 ± 0.828	2.45 – 6.08	4.22 ± 0.87	3.10 – 6.04	1.559	0.13
13	Bilibrubin (mg/dl)	0.0 - 1.0	0.56 ± 0.252	0.2- 1.1	0.57 ± 0.253	0.27- 1.03	0.356	0.724
14	AST (SGOT) (IU/L)	14642	25.95 ± 3.783	18.75 – 37.14	28.97 ± 8.234	21.2 – 62.3	2.092	0.045*
15	ALT (SGPT) (IU/L)	15008	23.80 ± 5.246	16.59 – 34.36	27.57± 11.175	14.1 – 74.5	1.75	0.091
16	Alk phosph (IU/L)	40 - 129	71.33 ± 18.5	44 - 118	71.37 ± 18.225	46 - 122	0.02	0.984
17	Na+ (mEq/L)	135 – 145	139.8 ± 3.145	132- 145	140 ± 3.09	134 – 147	0.819	0.42
18	K+ (mEq/L)	3.5 – 5.0	4.43 ± 0.350	3.8 – 5.4	4.45± 0.560	3.69 – 6.1	0.214	0.832
19	Cl- (mEq/L)	95 – 105	103.57 ± 2.84	96 – 109	104.03 ± 2.785	98 – 111	1	0.326

cycle): The women were asked to avoid sexual intercourse or use a vaginal product for 48 hours prior to collecting CVL for cytokines (interleukin [IL]-1b, IL-2, IL- 6, IL-10, IL-12, interferon -γ [IFN-γ] and tumor necrosis factor -α [TNF-α]) followed by colposcopy. CVL was collected by a non-traumatic 60 s wash, directing a stream of 10 ml PBS at the cervix using an intrauterine insemination catheter attached to a 12 ml syringe. The fluid pooled in the posterior fornix was aspirated and transferred to a 15 ml plastic conical tube and centrifuged. The supernatant was separated using a 0.22 micromillipore filter and stored at -80°C until analysis with BD OptEIA TM kits (USA). Colposcopy was performed with a digital video colposcope (Model: DVC 6000, Version 3.16E, Borze NY, USA), as per the CONRAD/WHO Manual for the Evaluation of Vaginal Products [18]. A 30 g 'Basant' cream tube was given and the women were taught to apply 3 ml intravaginally using an applicator. They were instructed to use 3 ml cream each night for the subsequent 14 consecutive nights and to wash and dry the plastic applicator for re-use. They were given panty liners, male condoms (for sexual intercourse at least twice a week) and a daily study record (DSR) form to note the time of cream use, intercourse and adverse events (AEs), if any. They were contacted telephonically 3-5 days later to check compliance. After 5-7 days, they returned to the clinic where DSRs and used tube were checked for adherence assessment. A speculum examination was performed to look for local inflammation. A second tube was given for the next 7 days. Follow-up visits: (i) On day 16-18 after initiating the use of cream (2-4 days after completing 14 days of cream), all baseline investigations (except CVL, colposcopy and Pap smear) were repeated. (ii) post-cream use CVL and colposcopy were done on day 18-20 after initiating cream or after the next menses i.e. on day 7-10 of the next menstrual cycle if these could not be done on day 18-20 due to onset of menses. (iii) The final visit was 6 -8 weeks after using cream for pelvic examination and Pap smear. The division of acquired immunodeficiency syndrome (DAIDS) toxicity table for Grading of Adult Adverse Experiences was used to characterize the severity of Adverse Events (AE) [19]. DSRs were reviewed at each visit to assess adherence, sexual activity and emergence of any symptoms. The used tubes were collected to further check compliance of cream use. Women who used cream daily were adherent. Those who experienced an AE that required cream discontinuation, but completed daily use preceding the AE were adherent. Non-adherent women (who missed cream for ≥3 days in the absence of AE) were asked to use the cream twice a day subsequently. Reasons for non-adherence were noted [20].

Cytokine assay in CVL was done by calculating the mean absorbance for each set of duplicate standards and samples. The mean zero standard absorbance was subtracted from each and standard curve was plotted on a log-log graph, concentration (in pg/ml) on x-axis and absorbance (in nm) on y-axis. A trend line was drawn through the standard points. To determine concentration of the unknown, the unknowns' mean absorbance value was found on the y-axis from the trendline equation. To check whether the cream interfered with cytokine assay, 3 ml cream and 10 ml PBS were vortexed for 10 min and centrifuged. The supernatant was filtered with Wattman filter paper number 30 (pore 0.45 microns) and serial dilutions made (undiluted,1:2 to 1:64). Taking PBS as a control, a known amount of cytokine was added to dilutions of cream solution and control to assay the cytokines.

Three questionnaires were employed to assess the acceptability of the cream. The initial questionnaire (for women on

Table 2: Summary of vaginal swab (wet mount & Gram stain) and urine examination results

Vaginal swab wet mount + gram stain)	Baseline (n=30)	Post -cream use (n=30)	P value
Normal findings	20 (66.7%)	26 (86.7%)	>0.05, NS
Abnormal findings	10 (33.3%)	4 (13.3%)	
Details of abnormal findings			
Candidiasis	3* (10%)	3 (10%)	
Trichomoniasis	none	none	
Bacterial vaginosis (BV)	8* (25.%)	1 (3.3%)	
Urine examination			
Normal findings	30 (100%)	29 (96.7%)	>0.05, NS
Abnormal findings	0	1 (3.3%)	

Table 3: Summary of self-reported symptoms, Colposcopic findings and Laboratory Adverse Experiences Judged Possibly Related to Study Products according to the Division of AIDS (DAIDS) table for grading the severity of adverse events

Adverse Event	Grade- 1 Mild	Grade- 2	Grade-3	Grade-4	Total (%)	Outcome
		Moderate	Severe	Potentially Life Threatening	N=30	
Self-Reported Adverse Experience Judged Possibly Related to Study Product						
Transient burning sensation and itching in vagina	9		-	-	9/30 (30%)	Resolved
Transient penile itching	3				3/30 (10%)	Resolved
Colposcopic Adverse Experiences						
Abrasion/erythema	1				1/30 (3.3%)	Resolved
Laboratory Adverse Experiences (hematology and biochemistry)						
Increased AST (SGOT) and	1	-	-	-	1/30 (3.3%)	Resolved
ALT (SGPT)						
Raised serum Potassium	1	1	-	-	2/30 (6.7%)	Resolved
Laboratory Adverse Experiences (urine examination)						
Positive urine fungal culture (candida albicans)		1	-	-	1/30 (3.3%)	Resolved
Laboratory Adverse Experiences (wet mount and gram stain of vaginal swab)						
Candidiasis	3	-	-	-	3/30 (10%)	Resolved
Intermediate Bacterial vaginosis	1	-	-	-	1/30 (3.3%)	Resolved

enrollment) had 10 questions with dichotomous responses (0 = non-acceptable, 1 = acceptable; maximum score = 10). It had questions regarding willingness of the woman and her husband to use the cream for 14 consecutive days and possible risks and benefits of the vaginal microbicide. The follow-up questionnaire (for women after 14 days of using the cream) had questions on applicator (any difficulty in filling the applicator, instilling the cream and re-using the applicator after washing and drying), vehicle (color, odor, packaging, amount and consistency of the cream) and use associated factors (ease of use, any leakage, need to use panty-liners, staining, interference with sexual activity) with dichotomous responses. The maximum score was 25 and higher scores indicated more acceptability. There were also questions on liked and disliked characteristics of Basant cream. The follow-up acceptability questionnaire (for men) was filled by women and their husbands' separately, it had five items with dichotomous responses, with a maximum score of 5. The questions were about the color, odor, any untoward symptoms, ease of use and interference with sexual activity. The questionnaires were adapted from acceptability studies of microbicides [20-23].

Sample size and statistics: With a sample size of 30, if toxicity occurred at a rate of 10%, the probability of observing at least two such events was 82%. If up to 10% participants were non-adherent, a subgroup analysis of adherent participants would provide 80% power to detect toxicity rates of at least 10%. Data was expressed using descriptive statistics (mean and standard deviation) for continuous variables. The paired 't-test' was used for intra-group comparison of means. For cytokine data, the Kolmogorov-Smirnov test showed the distribution to be non-normal. Hence, the Wilcoxon signed ranks test was applied. Statistical analysis was carried by SPSS version 13.0, free download (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412.)

Result

The mean age of women enrolled was 31.5±4.6 years (range 20-40) and parity was 2.07±0.6 (range 1-3). The majority (73.3% husbands' and 70% enrolled women) were educated up to class 10 or higher. Contraception was male condom (53.3%), sterilization (36.7%) and hormonal (10%). Hbs and VDRL were negative in all couples. Clinical examination, hematology, blood biochemistry, urine routine and culture were normal; urine for Chlamydia and Gonorrhoea by PCR were negative in all. The patch test with the cream did not show sensitivity in any woman. The mean vaginal pH was 4.1± 0.31 (range 4-5). The vaginal swab was normal in 20/30 (66.7%) and indicated infection in 10/30 (33.3%): candidiasis in 2, BV in 7 and candidiasis + BV in one. Pap smear was normal in 19/30 (63.3%) and inflammatory in 11/30 (36.7%); none had dysplasia. Women with vaginitis and inflammatory Pap smears were treated before enrolment. Colposcopy was normal in 28/30 (12 had cervical ectopy), two had acetowhite areas; which were biopsied and showed squamous metaplasia with no evidence of dysplasia. Post-cream use, pelvic examination was normal in 29/30; one had vaginal candidiasis. Hematology and blood biochemistry indices were similar to baseline values except for serum aspartate aminotransferase (AST) and creatinine, which were raised

Table 2: CVL cytokines (pg/ml) at baseline (A, follicular phase) and post-cream use (B, luteal phase) and post-cream use (C, follicular phase) in 11 women

N = 11										
Descriptive Statistics			Percentiles			Wilcoxon ranks test				
						Ranks				
cytokine	Mean ± SD	Range	25th	50th (median)	75th	Negative	Positive	Ties	Groups	P value
IFN -γ - A	0.21 ± 0.49	0 - 1.56	0	0	0	2	3	6	B - A	0.892
IFN -γ - B	0.29 ± 0.57	0 - 1.43	0	0	0.32	1	8	2	C - A	0.051
IFN -γ - C	1.02 ± 1.25	0 - 3.16	0	0.44	2.42	2	7	2	C - B	0.066
IL1- β - A	32.00 ± 35.61	0 - 101.1	0.19	23.53	59.41	8	2	1	B - A	0.059
IL1- β - B	7.03 ±14.84	0- 48.48	0	0	6.5	9	1	1	C - A	0.013
IL1- β - C	1.22 ± 2.69	0 - 7.41	0	0	0.21	5	1	5	C - B	0.046
IL 2 - A	87.12 ± 51.79	6.67 - 158.3	41.67	91.67	141.67	7	4	0	B - A	0.477
IL 2 - B	71.52 ± 61.99	0 - 169.0	15.67	64	149	9	2	0	C - A	0.016
IL 2 - C	22.91 ± 28.45	0 - 74.0	0	7.33	55.67	8	3	0	C - B	0.033
IL 6 - A	16.51 ± 32.83	0 - 102.61	0	0	31.45	2	9	0	B - A	0.041
IL 6 - B	52.75 ± 24.72	29.6 -109.27	35.6	46.93	59.93	2	9	0	C - A	0.01
IL 6 - C	49.36 ± 19.75	27.6 - 87.27	36.6	40.27	58.6	6	5	0	C - B	0.79
IL12 - A	215.15 ± 380.8	0 - 1345.83	65.83	107.5	209.17	8	2	1	B - A	0.059
IL12 - B	42.68 ± 58.89	0 - 152.5	0	1.2	97.2	8	2	1	C - A	0.013
IL12 - C	35.75 ± 74.79	0 - 255.57	0	8.43	31.29	6	3	2	C - B	0.374
IL10 - A	16.59 ± 17.84	0 - 46.88	0	14.53	32.76	5	6	0	B - A	0.155
IL10 - B	58.23 ± 71.38	0 - 223.28	0	35.28	102.5	5	2	4	C - A	0.091
IL10 - C	6.31 ± 13.09	0 - 41.68	0	0	10.08	6	2	3	C - B	0.05
TNF-α - A	2.34 ± 5.94	0 - 19.29	0	0	0	2	1	8	B - A	1
TNF-α - B	6.69 ± 22.21	0 - 73.67	0	0	0	2	0	9	C - A	0.18
TNF-α - C	0	0	0	0	0	1	0	10	C - B	0.317

(p=0.045 and 0.024, respectively), though the mean values were within the normal range (Table 1). Three women had minor abnormalities. Two women with serum potassium 4.16 and 4.51mEq/L respectively at baseline showed elevation post-cream use (5.91 and 6.1 mEq/L, respectively). A re-test showed levels of 4.62 and 4.95 mEq/L, respectively. This was Grade 1 toxicity in one (Grade 1 of DAIDS toxicity of serum potassium = 5.6 - 6.0 mEq/L) and Grade 2 toxicity in the other (Grade 2 = 6.1- 6.5 mEq/L) [19]. The third woman had fever upto 39° C on day three of cream use which came to 38°C next day and lasted for five days, but no other symptoms. She was evaluated by a physician who prescribed paracetamol and ciprofloxacin for 5 days. She continued to use the Cream for 14 days. Her baseline total leucocyte count, differential leucocyte count, platelets, AST and alanine aminotransferase (ALT) were normal (7900/mm3, P60, L34, M4, E2, 3.68 lac/mm3, 23.67 IU/L, 19.53 IU/L, respectively). On Day 16, these were 8000/mm3, P60, L34, M5, E1, 5.14 lac/mm3, 62.26 IU/L, 74.48 IU/L, respectively. A re-test 10 days later showed platelets, AST, ALT to be 5.41 lac/mm3, 31.72 IU/L, 36.20 IU/L respectively. A second re-test after another 7 days showed platelets to be 3.92 lakhs/ mm3. Her transaminitis was Grade 1 toxicity (Grade 1 toxicity for transaminases = 1.25-2.5 X ULN) [19]. Post-cream use, urine routine and culture were normal in 29/30, Chlamydia and Gonorrhoea was negative in all. In one woman (code 003), urine microscopy showed 6-8 pus cells/HPF and Candida spores. Bacterial culture was sterile but fungal culture grew Candida albicans. She received Fluconazole 100 mg BD for 10 days after a urology consultation. This was Grade 2 toxicity (as systemic treatment was needed). Urine microscopy and fungal culture were normal two months later. The mean vaginal pH was 4.03±0.18 (range 4-5) and vaginal swabs were normal in 26/30 (86.7%) and abnormal in 4/30 (13.3%). Of these four, 3 had candidiasis and one had BV. The Pap smear was normal in 24/30 (80%) and inflammatory in 6/30 (20%) (Table-2). Colposcopy was normal in 29/30. One woman (code 033) had vaginal candidiasis and cervical erythema (Grade 1 toxicity: Minimal cervical abnormalities: erythema, mucopurulent discharge, friability OR epithelial disruption < 25% of total surface) [19]. She gave history of sexual intercourse without condoms. She was given treatment for candidiasis, and advised condom use. At her next visit, there was no evidence of vaginitis or cervical erythema. Post-cream use, 9/30 (30%) women reported vaginal symptoms: 8 had mild irritation for about 5 min soon after applying the cream for the first one to 4 days, which subsided on its own; one had moderate irritation on day one, 3-4 h after cream application, which lasted for 1-2 h. She stopped using the cream on day 2 and 3 and visited the Hospital. Speculum examination was normal and she was advised to continue applying the cream. She again complained of mild irritation (now for initial 5 min after cream application) from days 4-7 but continued daily application. This symptom was not reported on the subsequent days. These 9 women were categorized as Grade 1 toxicity for vulvovaginitis (no or minimal interference with usual activities) [19]. The number of sexual contacts during the study were none in four, one each in four and >2 in 22/30.The mean number of sexual contacts in 26 couples was 2.81±1.8 (Range1-10), and all except two used condoms. Three husbands' had a single episode of transient penile irritation following sexual intercourse, two with condom use and one without condom, which subsided after washing with water. All AEs were mild to moderate and transient (Table 3). All women completed 14 days of cream use as per DSRs; 13/30 (43.3%) were completely adherent, 15/30 (50%) missed it for 1or 2 days each, one missed it twice for 2 days each (2 days due to vaginal irritation and 2 days for social reasons). Thus, 29 (96.7%) women were adherent. One woman missed to use the cream for 3 days in the absence of AEs and was considered non-adherent, though she used it twice daily on subsequent days. We assessed whether presence of cream in CVL interfered with cytokine assay (Figure 1). Assuming that

Table 5: CVL cytokines at baseline (A, follicular phase) and post-cream use (C, follicular phase) in 30 women

Descriptive Statistics			Percentiles			Wilcoxon ranks test			
cytokine	Mean ± SD	Range	25th	50th (median)	75th	Ranks			P value
						Negative	Positive	Ties	
IFN -γ -A	0.61 ± 1.81	0 - 9.49	0	0	0.17	4	18	8	0.01
IFN -γ -C	1.56 ± 2.16	0 - 9.09	0	0.63	2.54				
IL1- β - A	49.52 ± 53.23	0 - 184.04	0.92	28.25	87.16	23	4	3	0
IL1- β - C	7.89 ± 22.48	0 - 121.28	0	0	7.91				
IL 2 - A	109.39 ± 171.92	0 - 926.67	41.67	70.83	98.33	22	8	0	0.008
IL 2 - C	109.74 ± 339.37	0 - 1495.67	0	9.83	49.42				
IL 6- A	8.45 ± 21.05	0 - 102.61	0	0	7.61	2	28	0	0
IL 6- C	55.41 ± 25.43	24.6 - 126.6	37.6	46.1	70.1				
IL12- A	213.14 ± 318.62	0 - 1345.83	64.17	112.5	199.17	25	4	1	0
IL 12- C	109.74 ± 339.37	0 - 1495.67	0	9.83	49.42				
IL10- A	20.48 ± 24.45	0 – 95.71	0	12.76	32.91	17	7	6	0.076
IL10- C	22.32 ± 73.72	0 - 386.88	0	0	12.48				
TNF-α- A	16.05 ± 68.51	0 – 375.0	0	0	0	6	0	24	0.028
TNF-α- C	0	0	0	0	0				

the maximum amount of cream which could be present in the CVL would be 3 ml. A solution of 3ml cream in 10 ml PBS was prepared and serial dilutions made (undiluted,1:2, 1:4, 1:8, 1:16, 1:32, 1:64). A known amount of each cytokine was added to the serial cream-PBS solutions and PBS without cream (control). The amount of cytokines recovered from the cream-PBS solutions and PBS (control) were observed to be similar. Furthermore, when the cream-PBS solution was filtered, we needed a larger pore filter (0.45 μ) because the 0.22 μ filter used for CVL was unable to filter the cream-PBS solution as it was very viscous. Thus, filtration of CVL had removed most of the Basant cream from it and any residual cream did not interfere with cytokine assay. The levels of 7 cytokines (TNF-α ,IFN-γ, IL-1-β, IL-2, IL-6, IL-12, IL-10) were assayed in CVL in the follicular phase of the cycle (A, baseline, in 30 women), in the luteal phase of same cycle (B, post-cream use, in 11 women) and in the follicular phase of the next cycle (C, post-cream use, in 30 women). The "B" sample was collected between day 4 and 6 after last cream application (Day 18- 20 of cream initiation), as prior to that, too much cream was present in the vagina which made CVL collection technically difficult (Figure 2). The "B" sample could not be collected in 19 women due to onset of menses. The Wilcoxon signed ranks test was used to see for any change from C to A, C to B, and B to A (Table 4, Figure 3). The mean IL- 6 was

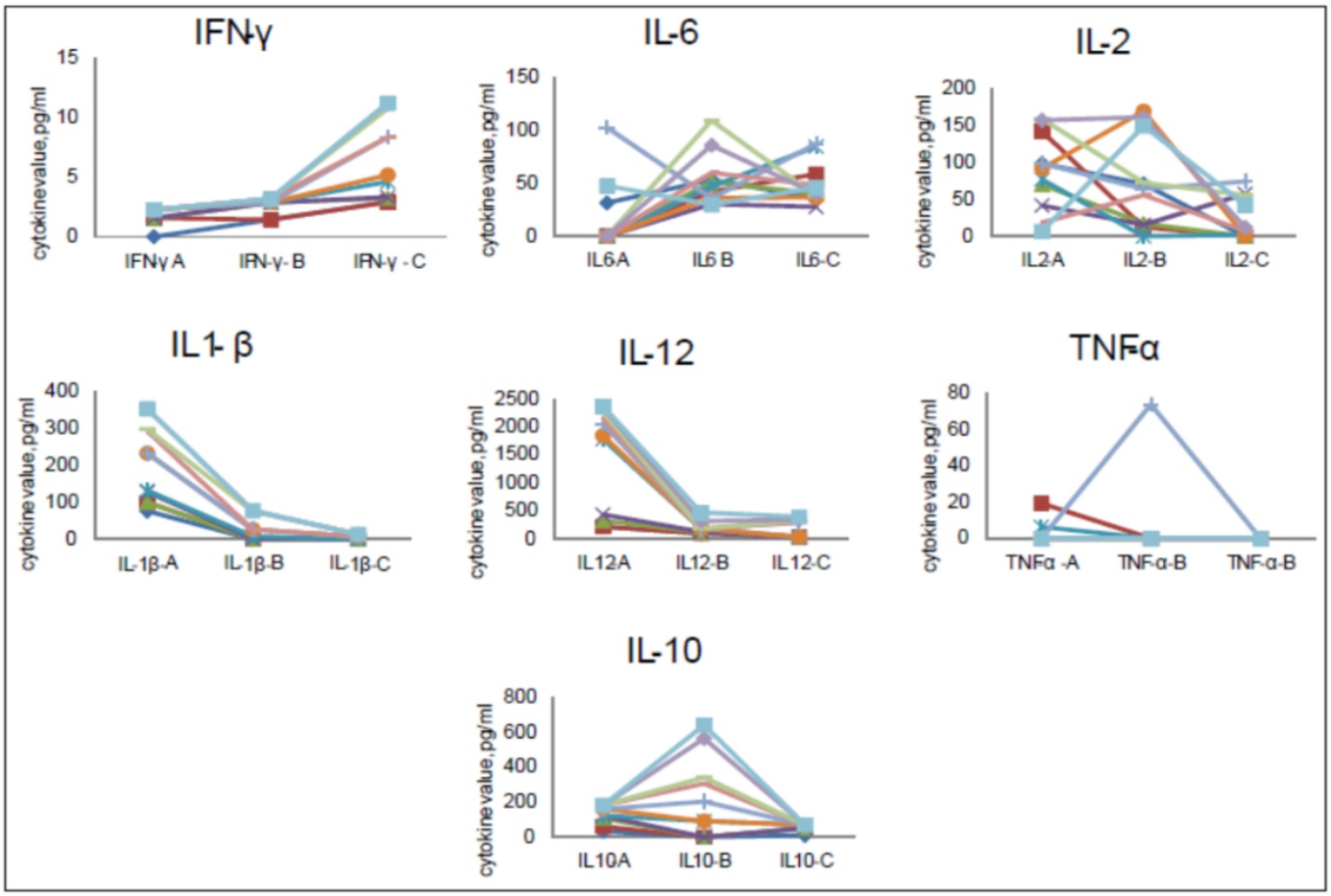

Figure 3: Graphs show individual woman's cytokine values in CVL samples A, B & C in 11 women

significantly elevated in the luteal phase (B) than at baseline (A) (52.75 ± 24.72 versus 16.51 ± 32.83 pg/ml, p=0.041). The mean IFN-γ and IL-6 were significantly elevated and the mean IL-1-β, IL-2, IL-12 and TNF-α were significantly lower in the next cycle follicular phase (C) when compared to baseline (A) (Table 5 and Figure 4). Each woman scored 10/10 in the initial questionnaire. They reported that they trusted the product because they were familiar with the health benefits of its herbal ingredients (amla, turmeric, rose water, reetha and A. vera) and felt that it may reduce the occurrence of vaginal infections. At follow-up, the women had a mean score of 21.83 ± 1.66 (range 19-25), showing a positive acceptability of 87.32%. Overall, 28/30 (93.3%) liked the cream's color, 29/30 (96.7%) found it to be fragrant, all were satisfied with its consistency and would choose a vaginal microbicide cream in future. However, need to use panty liners to prevent yellow stains due to curcumin was a matter of concern for 83.3% (25/30). The feedback of the 26/30 sexually active women regarding their husbands' perception showed a positive acceptability of 90.6% with a mean score of 4.53 ± 0.91 (range 1-5). Only 8 husbands' gave direct interviews and the mean score was 4 with a positive acceptability of 80%.

Discussion

Women constitute nearly half of the HIV infected population which they acquire chiefly by heterosexual transmission [24]. There is an urgent need to develop female controlled methods to prevent STIs as women in developing countries are usually unable to negotiate sexual practices. Vaginal microbicides use is one such method. The HIV virus can enter the intact vaginal epithelium and endocervix. Though vaginal epithelial cells have limited permeability to particles greater than 30 nm (HIV virion is 80-100 nm), the HIV virion enters the superficial epithelium by diffusing across a concentration gradient. Then it harbors on the surface of epithelial cells till it locates CD4+ and Langerhans cells, in which it then enters within 2–3 h. The HIV virion may survive within the Langerhans cells for about 3 days [25-29]. Some vaginal microbicides act by preventing viral attachment to epithelial cells, while others and increase lactobacilli and maintain an acidic pH and these have shown correlation with a decrease in HIV acquisition . Genital ulcers and mucosal trauma (e.g. by dry sex) increase susceptibility to HIV-1 [30-33]. Microbicidal formulations as gels or creams may help by minimizing the mucosal trauma.

The constituents of Basant cream are described in the ancient Ayurveda text: Bhavaprakash Nighantu or Indian Materia Medica of Sh. Bhavmisra (1600 AD, published by Chaukhamba Bharati Academy, Varanasi, India, 2004) and have been used individually as food or herbal medicines in India for many centuries. Turmeric's active component is curcumin. Its external application reduces skin inflammation. A decoction of E. officinalis (amla) helps to treat oral ulcers. External application of reetha extract is useful for reducing pain, inflammation, skin rash and itching. A. vera pulp applied topically helps in various skin diseases. Topical application of Rose reduces oral ulceration and inflammation. The present Phase I trial was carried out to test the safety and acceptability of Basant vaginal microbicide cream. This study was designed in accordance with recommendations for the clinical development of topical microbicides

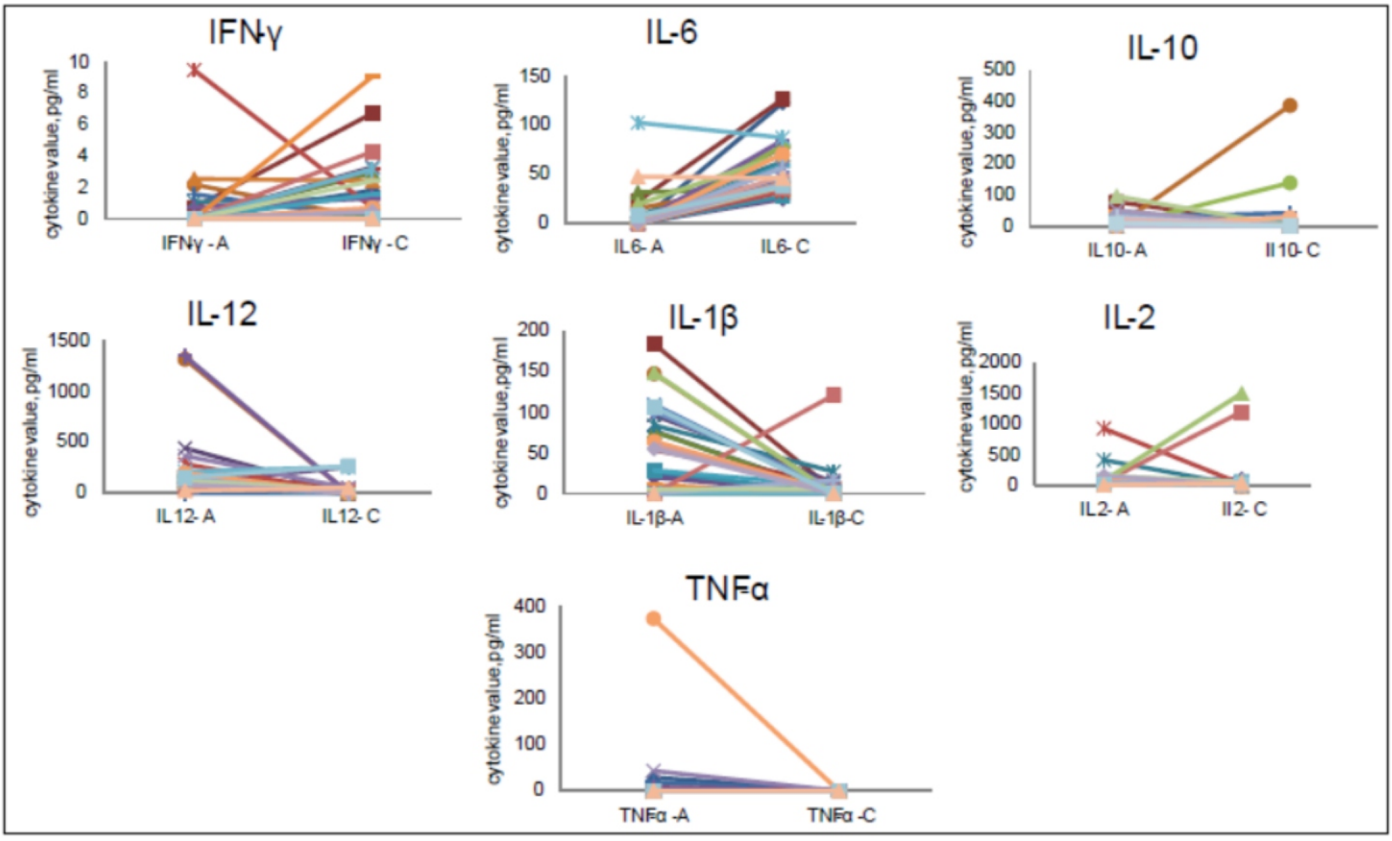

Figure 4: Graphs show individual woman's cytokine values in CVL samples A & C in 30 women

published by the International Working Group on Microbicides [34].

Overall, the results show Basant cream to be safe and well tolerated when applied intravaginally once a day for 14 consecutive days in 30 healthy women. The most common AE was mild transient vaginal irritation in 30% women and transient penile irritation in 10% of the male partners'. All AEs were mild and resolved within the study period. The present data indicates the lack of any significant side effects on metabolic and organ functions. Furthermore, this cream maintains an acidic vaginal pH and caused no significant change in vaginal microflora. It disperses well and remains in the vagina for 24-36 hours, an observation which may result in the need for less frequent applications.

However, the post-cream use elevated pro-inflammatory cytokines in CVL (IL-6 and IFN –γ), which is a cause for concern. Post-cream use, the values of both IL-6 and IFN-γ were significantly elevated in the next cycle follicular phase as compared to baseline in all 30 women. Furthermore, the values of IL-6 were significantly elevated in the same cycle luteal phase as compared to baseline in 11 women. However, post-cream use, the values of IL-1- β, IL-2, IL-12 and TNF-α were significantly lower in the next cycle follicular phase as compared to baseline, and IL-10 also showed a similar trend. IL-1- β and IL-6 levels have been reported to be 5 fold higher in the follicular phase than in the luteal phase of a normal menstrual cycle [35]. Therefore, the elevated IL-6 level in the luteal phase may be even more significant, considering that it is expected to be 5 fold lower. Increased level of IL-1, IL-6, and IL-8 in the vaginal secretions are sensitive indicators of compound-induced mucosal toxicity and are a valuable tool in identifying vaginal microbicides that may enhance, rather than decrease, HIV transmission [36]. On the other hand, the increase in IL-6 may not necessarily be a negative feature of the product. IL-6 is critically important for acquired immunity and its slight increase after Basant cream may be beneficial. IFN -γ is also increased and this is beneficial for anti-viral defenses. Both these changes may not be considered as a sign of pro-inflammatory activity as at the same time this cream did not increase IL-1- β as N-9 and other irritating products do [37]. Rather, it resulted in decreased IL-1- β in all women. Whether the post-cream use raised IFN-γ and IL-6 values reflects a pro-inflammatory effect will need to be observed carefully in the future in order to assess its impact on vaginal mucosal inflammation and, consequently, susceptibility to HIV infection.

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