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BASANT, a Polyherbal Safe Microbicide Eliminates HPV-16 in Women with Early Cervical Intraepithelial Lesions

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Abstract

Carcinoma cervix is a major cancer of women killing 510,000 women every year worldwide. Human papilloma virus (HPV) infection of cervical cells initiates the transformation of the cervical cells to malignant stage. HPV-16 is the most frequent type of HPV causing these changes. We report here the elimination of HPV-16 from the infected cells of all (11/11) women positive for HPV-16 by 30 intra-vaginal intakes of BASANT.

Keywords

Carcinoma Cervix, Human Papilloma Virus, Microbicide BASANT

1. Introduction

Carcinoma cervix is a major killer of women due to cancers. It is the second largest cancer of women worldwide, with 510,000 new cases and 288,000 deaths annually [1]. Every year, almost 74,000 women die of cervical cancer in India, which are a little more than one fourth of the deaths due to this cancer in the world [2]. This cancer is caused by infection of cervical cells by human papilloma virus (HPV), a discovery, for which Nobel Prize was given to Harald zur Hausen. Amongst the various HPV strains, the most frequent causing eventually carcinoma cervix are HPV 16 & 18 [3]. The progression of infected cervical cells to cancer stage is slow and goes through

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various stages recognized as cervical intraepithelial neoplasia CIN-1 CIN-2 and CIN-3. Pap smear is routinely employed as test to detect infection of the cervical cells. Gardasil (Merck) and Cervarix (Glaxo Smith Kline), two good vaccines have been made, but these generate antibodies which can prevent the entry of the virus, and are thus useful before the woman has had sexual intercourse. The antibodies generated by these vaccines fail to eliminate the virus from the infected cervical cells. We report here not only the ability of a microbicide BASANT which prevents the entry of HPV in cervical cells, but also its capability to eliminate HPV 16 from infected cervical cells at the early stage CIN-1 (low grade squamous intraepithelial lesion LSIL).

2. Clinical Trial Product BASANT

BASANT was formulated with 95% pure diferuloyl methane (E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), (Curcumin) from *Curcuma longa*, purified extracts of *Emblica officinalis*, Neem (*Azadirachta indica*) leaves, and *Aloe vera* (*Aloe barbadensis*). These ingredients were dispensed along with pharmacopoeially approved excipients: citric acid, sorbitol, microcrystalline cellulose, sodium starchglycolate, starlac, crospovidones and sodium alginate as a lubricating agent. It has wide spectrum action against a variety of pathogens such as *Neisseria gonorrhoeae*, including strains resistant to penicillin, tetracycline, nalidixic acid and ciprofloxacin [4]. 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 [4]. It exercises also inhibitory action on free as well as cell-infected *Chlamydia trachomatis* [5]. It prevents the entry of HPV 16 in Hela cells[4]. It is also effective against both CCR5 and CXCR4 tropic HIV-1 lab-adapted strains and primary isolates of HIV of different clades [6].

3. Study Design

3.1. Enrollment

A total of 159 women coming to Obstetrics & Gynecology clinic of Jawaharlal Nehru Medical College Aligarh were examined clinically by visual inspection of cervix and by staining with acetic acid (VIA). Cervical scrapes were collected using Ayre's spatula by rotating it at 360˚ around the circumference of the ecto-cervical region and endo-cervical canal and Pap smears were prepared. Scraped cells were suspended in cold PBS (phosphate buffered saline) for isolation of DNA. Women enrolled were in reproductive age group (25 - 45 years), employing contraceptives, had regular monthly menses every 21 - 35 days, or amenorrhea due to hormonal contraceptive use, and were agreeable to abstain from non-steroidal anti-inflammatory drugs (NSAIDS) during the study period. Women having suspicious lesions, and having persistent vaginal discharge were also included. The exclusion criteria were: women who have undergone total hysterectomy, or are using intrauterine contraceptive device (IUCD) as a form of birth control method and who are pregnant or lactating. All women enrolled were sexually active, and their partners were willing to comply with the use of the barrier method of contraception during the study period. On the basis of inflammatory cervix & abnormal Pap smear, 35 subjects were enrolled for treatment

Treatment

Patients enrolled were assigned alternatively to receive either intravaginal capsules of BASANT, two capsules (250 mg each) each night or two Placebo capsules for 30 days, excluding the days of menstrual period and they were evaluated for the presence or absence of HPV16 in cervical cells by generic and type specific PCR. Pap smears were also examined within three days of the completion of the treatment. HPV DNA presence or absence was tested as primary outcome. Pap smear, visual inspection and colposcopic examination of the cervix were carried out before and after the treatment, to study the effect of treatment on the lesion and on cytology as outcome.

3.2. DNA Extraction and Analysis for Presence of HPV-16 Infection

High molecular weight genomic DNA was extracted from cervical scraps of patients prior to and after completion of the treatment by the standard method of Proteinase K digestion and phenol chloroform extraction procedure. PCR amplification was performed using the consensus degenerate primers (MY09: 5'-GCMCAGGGW CATAAYAA TGG-3', MY11: 5'-CGTCCMARRGGAWACTGATC-3' where M = A/C; W = A/T; Y = C/T; R = A/G) as described earlier[8]. Further typing of high risk HPV type 16 was done by type specific primers. β globin

Table 1. Pre and post treatment with BASANT of HPV-16 positive patients.

			PRE TREATMENT		POST TREATMENT	
S.N.	**AGE**	**PARITY**	**PAP**	**HPV-16**	**PAP**	**HPV-16**
1.	42	3 + 0	LSIL	+VE	INF	NEGATIVE
2.	27	4 + 0	LSIL	+VE	INF	NEGATIVE
3.	35	3 + 0	INF	+VE	NORMAL	NEGATIVE
4.	28	1 + 1	INF	+VE	INF	NEGATIVE
5.	45	3 + 0	INF	+VE	INF	NEGATIVE
6.	35	4 + 0	INF	+VE	INF	NEGATIVE
7.	30	2 + 1	LSIL	+VE	INF	NEGATIVE
8.	45	2 + 0	LSIL	+VE	INF	NEGATIVE
9.	38	5 + 2	INF	+VE	INF	NEGATIVE
10.	35	3 + 1	AGUS	+VE	INF	NEGATIVE
11.	38	3 + 1	INF	+VE	INF	NEGATIVE

Number of patients screened for HPV DNA: 159; Number of patients positive for HPV-16: 19; Intravaginal Basant given with written consent: 11 HPV positive patients accepting to take Basant; HPV-16 turning negative after intravaginal Basant: 11; Intravaginal Basant given in patients with abnormal cytology: 10; Abnormal cytology after treatment: 00.

gene was used as internal control. PCR was performed in a 25 µL reaction mixture containing 50 - 100 ng DNA, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 125 µM of each dNTPs (dATP, dGTP, dCTP, dTTP), 5 pmoles of oligonucleotide primers and 0.5 u Taq DNA Polymerase. The temperature profiles used were initial denaturation at 95˚C for 4 min followed by 30 cycles of denaturation at 95˚C for 30 s, annealing at 55˚C for 30 min and extension at 72˚C for 1 min which was extended for 5 min at the final cycle.

3.3. Observations

Out of 35 subjects enrolled, 19 patients were found positive for HPV-16. Only 11 out of these 19 agreed to undergo intravaginal treatment with BASANT. After 30 days of intravaginal insertion of BASANT, all eleven HPV-16 positive cases became HPV negative. Pap smears became normal for case no 3 and was inflammatory in other cases. The findings are summarized in **Table 1**.

4. Discussion

The ability of polyherbal microbicide BASANT which not only prevents the entry of HPV in Hela cells[4] but also eliminates HPV-16 from already infected cells reflects a valuable property of this microbicide. We had earlier made a microbicide named “Praneem” which had undergone similar trials in women at early stage of HPV 16 infection as reported elsewhere [7]. After 30 days of use of Praneem, 6 out of 10 subjects showed the elimination of HPV 16. A repeat treatment of 4 patients who had persisted HPV 16 cleared HPV in two more cases. Those receiving placebo had shown clearance in only 1 out of 10 cases. A disadvantage of Praneem was the irritation that it caused in some subjects presumably because of the presence of saponins from *Sapindus mukorossi* in the previous formulation. When formulating BASANT, such saponins were avoided. A phase II clinical trial with BASANT was carried out in 20 women suffering from recurring episodes of vaginosis. Seven intravaginal intakes of BASANT cleared vaginosis in 14 out of 20 patients. None of the 20 women experienced any irritation. Acceptability of BASANT was high. A combination of 3 selected probiotics along with BASANT relieved vaginosis in 19 out of 20 women restoring fully healthy vagina with acidic pH [8]. The present trial shows another capability of this formulation to eliminate HPV-16 from cervical cells at early stages of low squamous intraepithelial lesion (LSIL).

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