

Selective Promotion of Protective Lactobacilli by Gynovash Foam Cleanser to Maintain Healthy Vaginal Ecosystem

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Abstract

Background and objective: Appropriate intimate feminine hygiene can relieve the discomforting symptoms of abnormal vaginal discharge, bad odour, itching, dryness and improves overall well-being.

Present study aimed to investigate efficacy of Gynovash (Millennium Herbal Care Ltd.) foam cleanser for the preliminary evaluation of anti-microbial activity and selective growth promotion of protective Lactobacilli to uphold an optimum vaginal ecosystem.

Methods: The *in-vitro* microbial activity of Gynovash foam cleanser was assessed by agar well diffusion method and time-kill determinations for *Candida albicans* and *Lactobacillus plantarum*.

Results: Gynovash foam cleanser initially inhibited growth of both organisms with zone of inhibition 18.7 ± 1.5 mm for *Candida albicans* and 19.8 ± 1.2 mm for *Lactobacillus plantarum*. Time kill determination study revealed that with increase in contact time, Gynovash promoted growth of *Lactobacillus plantarum* while the growth of *Candida albicans* remained inhibited. These results validated the product mechanism that it kills all pathogenic and non-pathogenic organisms initially, and later with time it selectively supports the growth of Lactobacilli which could suppress pathogens and helps maintaining vaginal ecosystem.

Conclusion: Gynovash foam cleanser, a sulphate free, silicon free phyto-constituent enriched product could preserve the overall health of vaginal ecosystem.

Keywords: Personal; Health; Gynovash; Hygiene; Intimate care; Gynaecology; Feminine; Microbial; Vaginitis; Pathogen; Organism

Introduction

The vaginal ecosystem is dominated by a non-sporing gram-positive bacilli, Lactobacillus, which keep the environment acidic (pH 3-4) by secreting lactic acid [1].

Moreover, these Lactobacilli maintain healthy vaginal ecosystem by competing with other bacteria including some pathogens for epithelial cell receptors and also via generation of growth inhibiting compounds collectively with innate host defenses.

An alteration of vaginal lactobacillus populations has been shown to be associated with increased risk of bacterial vaginitis, yeast vaginitis, urinary tract infection, unpleasant odours, vaginal irritation and discomfort [2].

Although the structural and functional aspects of vaginal environment are well reported in literature, the impact of personal hygiene practices on the same is not well recognized [3].

Personal choice and social practices significantly impact feminine hygiene practices. Improper practices such as washing with plain water

or using preparation with harsh surfactant may cause abrasion and be harmful to local vaginal flora increasing risk of infection.

The clinical benefits of vaginal douching have been controversial. They may alter the normal vaginal flora and subvert the innate immune system making them vulnerable to infections [4-6]. Conforming to this fact, in an Iranian study of 500 women, poor menstrual and vaginal hygiene practices were associated with bacterial vaginosis [7].

This emphasises a need for safe and hygienic practices for women worldwide. Routine washing of the vulva is advisable to avert accumulation of vaginal discharge, urine, sweat, and also for preventing fecal contamination.

Appropriate intimate feminine hygiene can support avoidance or relieve the wearisome symptoms of abnormal vaginal discharge and itching and improve overall well-being. In a large clinical study, a 4-week use of feminine wash products developed from natural plant extract improved symptoms and quality of sexual activity and corrected the vaginal pH [8,9].

Taken together, these data demonstrate the importance of appropriate female intimate hygiene using properly designed and evaluated products with key attributes including hypoallergenic, soap-

free, pH friendly, non-irritating, protecting dryness, and helping maintain balanced microflora.

The present study was undertaken to evaluate in-vitro efficacy of Gynovash foam cleanser against pathogenic microorganisms and promoting selective growth of protective Lactobacilli to create an optimum environment for growth of beneficial flora.

Material and Methods

The *in-vitro* microbial activity of Gynovash foam cleanser was conducted at Bhavan's Research Centre Microbiology, NABL (ISO, IEC 17025:2005) accredited laboratory.

The following methods were used:

- Agar well diffusion method
- Time-kill determinations

Test organisms

Two standard test organisms, *Candida albicans* (ATCC 10231) and *Lactobacillus plantarum* (ATCC 8018) were selected and procured as representative of pathogenic yeast and resident Lactobacilli microflora of vagina.

Cultures were then subcultured and maintained using Lactobacillus MRS agar (Himedia) and Sabouraud's dextrose agar (SDA) (Himedia).

Inoculum preparation

Inoculum was prepared by inoculating in 0.5ml of diluted culture in agar medium to achieve 1.0×10^6 CFU/ml deliveries.

Agar well diffusion method

An SDA plate was inoculated with *Candida albicans* culture by spreading on the surface of the media. An 8-mm well was made in the center of the medium on each plate and 0.2 ml of sample [2 self-foaming dispensation of Gynovash (test) versus control] was added.

Both Lactobacillus MRS agar and SDA plates were incubated at $30^\circ\text{C} \pm 1^\circ\text{C}$ for 48 hours under micro aerophilic conditions.

The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (mm). Plating was performed in triplicates to derive mean and standard deviation.

Time-kill determinations

For Test Sample preparation 2 pumps of the test sample [2 self-foaming dispensation of Gynovash] was sprayed in 2 sterile 100 ml bottles separately. 100 ml of sterile MRS broth and SDB was added to the respective bottle.

Simultaneously control set was prepared. 1ml of each diluted suspension of *Candida albicans* and *Lactobacillus plantarum* was added to control and test set to achieve 1×10^5 CFU/ml.

The test and control set for *Lactobacillus plantarum* and *Candida albicans* was checked quantitatively at different intervals such as at 0, 6, 24, 30 and 48 hours by surface spread technique.

During the contact time the test and the control set were incubated at $30^\circ\text{C} \pm 1^\circ\text{C}$. Plates were checked and counted for significant growth of test organisms.

Results

The zones of inhibition for *Candida albicans* and *Lactobacillus plantarum* were 18.7 ± 1.5 mm and 19.8 ± 1.2 mm respectively.

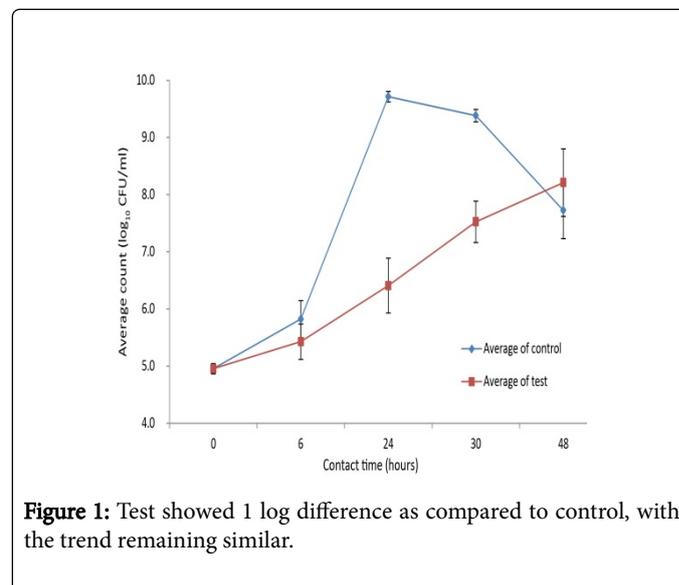


Figure 1: Test showed 1 log difference as compared to control, with the trend remaining similar.

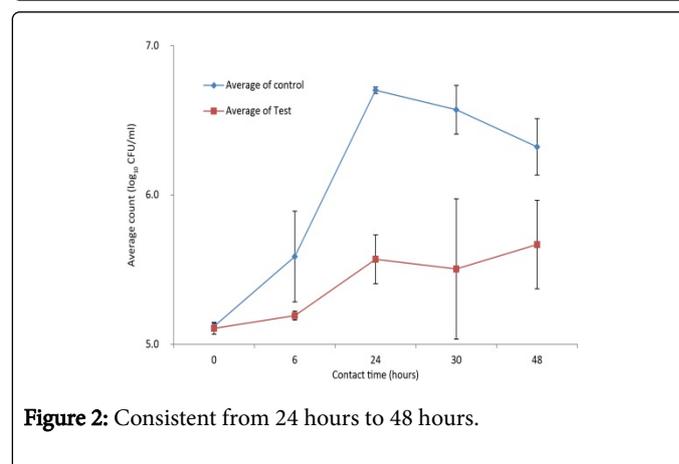


Figure 2: Consistent from 24 hours to 48 hours.

Test showed 1 log difference as compared to control, with the trend remaining similar and consistent from 24 hours to 48 hours (Figures 1 and 2).

The results of time-kill determinations are shown in Figure 1 and 2. The \log_{10} CFU/ml of *Lactobacillus plantarum* in control was 4.95 ± 0.09 which increased up to 9.71 ± 0.094 at 24 hours.

The growth of *Lactobacillus plantarum* in control achieved plateau through 24 hours to 48 hours with mean \log_{10} CFU/ml of 9.38 ± 0.1 at 30 hours and 7.72 ± 0.49 at 48 hours.

On the contrary, at 24 hours of contact time, the growth of *Lactobacillus plantarum* was inhibited (6.41 ± 0.48 \log_{10} CFU/ml) from baseline (4.95 ± 0.04 \log_{10} CFU/ml) with test product as compared to that of control (9.72 ± 0.09 \log_{10} CFU/ml).

However, *Lactobacillus plantarum* continued to grow logarithmically with test product as compared to the control at 30 hours (7.52 ± 0.36 \log_{10} CFU/ml) and 48 hours (8.21 ± 0.59 \log_{10} CFU/ml) as shown in Table 1.

| Test Organism | Test/Control | Mean Counts (Log 10 CFU/ml ± Standard Deviation) | | | | |
|--------------------------------|--------------|--|-------------|-------------|-------------|-------------|
| | | 0 hour | 6 hours | 24 hours | 30 hours | 48 hours |
| <i>Lactobacillus plantarum</i> | Control | 4.95 ± 0.09 | 5.82 ± 0.32 | 9.72 ± 0.09 | 9.38 ± 0.11 | 7.73 ± 0.49 |
| | Test | 4.95 ± 0.04 | 5.43 ± 0.31 | 6.41 ± 0.48 | 7.52 ± 0.36 | 8.21 ± 0.59 |
| <i>Candida albicans</i> | Control | 5.12 ± 0.02 | 5.65 ± 0.30 | 6.70 ± 0.02 | 6.59 ± 0.16 | 6.35 ± 0.19 |
| | Test | 5.13 ± 0.04 | 5.21 ± 0.03 | 5.57 ± 0.16 | 5.60 ± 0.47 | 5.79 ± 0.30 |

Table 1: Mean counts for contact-kill determination study.

Discussion

The zone of inhibition for *Candida albicans* and *Lactobacillus plantarum* indicated antimicrobial activity of Gynovash foam cleanser against both the organism. The growth of *Candida albicans* was low compared to *Lactobacillus plantarum* as shown in the table 1. However, the test showed 1 log difference as compared to control, with the trend remaining similar and consistent from 24 hours to 48 hours (Figures 1 and 2).

The results show that the product initially inhibited growth of both organisms; however, with increase in contact time, it allowed growth of *Lactobacillus plantarum*, while *Candida albicans* remained inhibited. These findings validate the product mechanism that it kills all pathogenic and non-pathogenic organisms initially, and later with time it selectively supports the growth of *Lactobacillus plantarum* in population that suppress pathogens.

Aloe vera based preparations have been shown to inhibit growth of *Candida albicans* [10,11]. Gynovash comprises of potent anti-oxidants, anti-inflammatory, antibacterial, anti-mycotic phyto-constituents (Neem leaf oil, Clove oil, Tea tree oil) and lactic acid which acts on the vaginal skin epithelial cells interfering adhesion of fungal and bacterial microbes to vaginal epithelium cells and further support and accelerate growth of Lactobacilli.

The foaming nature of Gynovash may be responsible for better absorption and quick penetration, can be applied and spread more easily onto large areas without leaving any greasy residue over surface.

The area of intimate feminine hygiene has not received enough attention in the medical literature, thus making education a priority. In 2011, the Royal College of Obstetricians and Gynecologists (RCOG) performed extensive literature searches to develop evidence-based guidelines intended for the general gynecologist for improving initial assessment and care of vulvar skin disorders [12]. Similarly, a committee from the Middle East and Central Asia (MECA) conducted extensive literature searches to form recommendations on female genital hygiene. Both guidelines suggest daily vulva cleansing with a gentle hypoallergenic liquid wash [13].

Conclusion

In summary, proper female intimate hygiene using appropriately designed products is desirable for overall feminine well-being. Findings from the current study suggests that Gynovash, a sulphate free, silicon free product containing lactic acid with blend of antimicrobial natural essential oils, soothing and moisturizing benefits of Aloe vera and Vitamin E may be beneficial for selective growth of lactobacilli to keep its vaginal pH in the acidic range and thus, keep the balance of bacteria, yeast and overall health of vaginal ecosystem in order.

Conflict of Interest

Authors declare no conflict of interest.

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