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Safety Study of Three Concentrations of UniPron Vaginal Contraceptive Microbicidal Gel

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Abstract Background: UniPron is an antimicrobial and a spermicidal agent that contains citric acid as the active component. This study was designed to evaluate the effects of three different concentrations of UniPron in the baboon (*Papio anubis*) model. Methods: Twenty sexually mature female baboons were used in this study. Vaginal pH and microflora, blood chemistry, vaginal and cervical histology were evaluated at baseline and after administration of 15 ml of UniPron 0.4, 0.8 and 1.2 gm or placebo twice a week for eight weeks to each randomized treatment group. Results: Baseline vaginal pH was 5.2±0.8. There was no significant difference in the vaginal pH and blood chemistry parameters. The microflora composition was diverse and slight variation in percentage frequency between UniPron concentrations was observed. No detectable histological changes were observed in the vaginal or cervical sections. Conclusion: Repeated application of 15 ml of three UniPron concentrations appeared to be safe when administered intravaginally in the baboon model.

Keywords: *UniPron, baboon, safety, vagina, microbicide, contraceptive*

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1. Introduction

The role that the vaginal bacteria plays in protecting the host from sexually transmitted infections (STIs), including HIV infection is becoming increasingly appreciated. Due to increased incidence of STIs, the development of vaginal products with microbicidal activities is a high priority in contraception research [1]. Women who are at risk of STIs are by definition also at risk of unintended pregnancy [2]. In the recent past, research and development of microbicidal contraceptives has been widely considered as they would ideally provide a convenient, readily available method of self-protection against STIs in addition to preventing unplanned pregnancy [3,4,5]. This female-controlled method of protection would also empower women because it offers a system that does not require consent from men. However, a major challenge has been to design mechanism-based products that are highly effective against pregnancy and STIs/HIV infections while lacking detergent-type effects on epithelial cells and normal vaginal flora [6]. Over the past two decades, both contraceptive and non-contraceptive microbicidal vaginal products have been undergoing development and investigation [4,5,7-13]. There is concern over the vaginal and cervical irritation caused by some of the products under investigation because these epithelial changes are thought to increase the risk of STI/ HIV acquisition for women at high-risk for infection, especially with frequent application [14,15,16,17]. It is essential that the evaluation of the potentially harmful effects of these compounds on the vaginal epithelium and/or normal vaginal flora be made before subjecting women to their use. Effective multipurpose prevention technologies for women's reproductive health is an area searching for innovative strategies and approaches to increase access and adherence [2]. Research and development of dual microbicidal contraceptive are yet to produce safe and effective products and new approaches continue to emerge to achieve this goal. This study aimed to assess the safety of vaginal dosing of 0.4 gm, 0.8 gm and 1.2 gm of UniPron in terms of local and systemic effects in the baboon model. Smugel gel whose safety profile on the parameters to be assessed has been established [18] was used as a placebo.

2. Materials and Methods

2.1. Animals

Twenty healthy sexually mature cycling female olive baboons used in this study. The animals were housed at the Institute of Primate Research (IPR), Nairobi, Kenya which is a WHO Collaborating Centre that ethically utilizes non-human primates to improve human health and is guided by international and local standards including Primate Vaccine Evaluation Network, the Council for the International Organizations of Medical Sciences and the National Institutes of Public Health Service policies. The

baboons were fed on a commercial monkey cubes (Unga Feeds Ltd, Nairobi, Kenya) supplemented with fruits, vegetables and water *ad libitum*. All animal procedures and care were conducted in accordance with internationally accepted standard operating procedures. Prior approval for use of baboons was obtained and sample collection performed following approved Institutional Review Committee (IRC) protocols.

2.2. UniPron

UniPron is an antimicrobial and spermicidal agent that contains citric acid as the active compound. It is a clear fluffy, acidic, water based and non-detergent lubricating gel. The product is heavily buffered with 0.4 gm, 0.8 gm and 1.2 gm concentrations of citric acid having a pH of 3.47, 3.02 and 2.7 respectively. It is composed of Carbomer, sodium benzoate, sodium carboxymethyl cellulose, EDTA, disodium hydrogen phosphate, purified water and citric acid. The buffering agent in UniPron is carbomer (carboxyvinyl polymers of high molecular weights). Its therapeutic classification is antifertility and/or microbicide agent. The product is stable at both room (approximately 22°C) and body temperature and has a shelf life of 24 months. UniPron's mechanism of action is by lowering vaginal pH to 3.5 immediately after administration and maintaining the acidity for about 3 hours. It has the ability to preserve an acidic vaginal microenvironment due to its highly buffered low pH. Smugel gel, whose safety profile has been evaluated in the baboon model [19] was used as a placebo.

2.3. Study Procedures

Samples were collected from all the animals to obtain baseline data of vaginal pH, vaginal flora, and blood chemistry, vaginal and cervical mucosal integrity. Thereafter, the animals were randomized 1:1:1:1 to receive placebo (Smugel), UniPron 0.4 gm, 0.8 gm or 1.2 gm. Pre-clinical safety studies of intravaginally administered UniPron were performed in baboons to closely mimic the intravaginal application of a vaginal microbicidal contracetptive gel in women. The packaged 15 ml of the study gels in prefilled single use applicators were applied into the vagina of each animal twice a week for eight weeks, followed by sample collection during the entire treatment period and after the treatment. Vaginal and cervical biopsies were collected at baseline and at the end of vaginal gel application. Before any vaginal insertions for pH assessment, sample collection or gel application, the vulva was disinfected using betadine.

2.4. Vaginal pH

Vaginal pH was assessed for five weeks before treatment to obtain baseline data. Thereafter the assessment was done every week during the eight weeks treatment period with Smugel and UniPron, and one week post treatment. The pH was assessed twice a week as previously described [18].

2.5. Clinical Chemistry

Ten ml of blood was collected from all the animals before treatment to obtain baseline data. During treatment, blood was collected from each group at week two, four, six, eight, ten (two weeks post treatment) to obtain serum samples for subsequent chemistry assays to evaluate systemic toxicity. The aliquoted sera were used for analysis of total protein (TP-g/dl), albumin (ALB-g/dl), urea (Ure-mg/dl), creatinine (CRE-mg/dl), aspartate aminotransferase (AST-U/L), alanine aminotranferase (ALT-U/L), alkaline phosphatase (ALP-U/L) and total bilirubin (TBIL-mg/dl) as previously described [18].

2.6. Vaginal Microbiology

Vaginal swabs were collected once a week for five weeks from all the animals to establish baseline data. Thereafter, the swabs were collected from each of the four treatment groups during the entire period of gel treatment and one week post treatment. The vaginal swabs were processed and vaginal flora determined from Gram-vaginal smears, vaginal culture and biochemical tests as previously described [18].

2.7. Vaginal and Cervical Biopsies

Every time application of both Smugel and UniPron was done and before collection of biopsies, there was physical examination of the vagina and the cervix to assess any signs of mucosal irritation. Vaginal biopsies were collected once from each animal before treatment to obtain baseline data and at the end of the product application, processed and stained as described [19]. Cervical biopsies were collected at the same time by using an endoscopic cup (Karl Storz, GmbH & Co. KG, Germany) to pinch the epithelium at the external os of the cervix and processed in a similar version.

2.8. Statistical Analysis

Analysis of vaginal pH, serum chemistry and microflora percentage frequency isolation was done using StatView software (version 5.0, SAS Institute Inc, Cary, NC, USA) and the data were expressed as mean±SD ($\overline{x}$ ± SD). Differences with P values >0.05 were considered not significant at 95% confidence interval (CI).

3. Results

3.1. Baboon Vaginal pH Evaluation

Vaginal pH at baseline was determined to have a mean±SD of 5.2±0.8 (data not shown). The variation observed in vaginal pH during the eight weeks treatment was not statistically significant (P>0.05) across all gel arms (Table 1). Similarly, no significant difference was observed in vaginal pH one week post application.

3.2. Blood Chemistry Parameters

The parameters evaluated included TP, ALB, URE, TBIL, CRE, ALP, ALT and AST. The differences observed across all gel arms were considered not significant (P>0.05) (Table 2). Similarly, no significant differences were observed two weeks post gel application.

Table 1. Vaginal pH of animals treated with Smugel (Placebo) or UniPron($\bar{x}$ ± SD). The pH was evaluated during weekly treatment with Smugel or UniPron and one week post treatment

Vaginal gel	Sample points (weeks)								
	1	2	3	4	5	6	7	8	9
Smugel (Placebo)	5.6±0.8	5.5±0.3	6.0±0.6	5.6±0.5	5.9±0.5	6.2±0.7	5.6±0.5	5.6±0.8	5.7±0.9
UniPron 0.4 gm	5.3±0.6	5.2± 0.5	5.3±0.7	6.1±0.8	5.6±1.0	4.6±0.7	5.6±1.0	5.6±0.8	6.0±0.7
UniPron 0.8 gm	5.6±1.0	6.5±1.0	6.0±0.9	4.9±0.9	5.6±0.5	5.5±0.9	6.0±1.0	5.6±0.9	4.7±0.9
UniPron 1.2 gm	5.4±0.6	5.2±0.5	5.7±0.6	5.7±0.7	6.2±0.7	5.7±0.8	6.1±0.8	6.3±0.8	5.6±1

Table 2. Blood chemistry profiles at baseline, during treatment period with Smugel or UniPron and at two weeks post treatment ($\bar{x}$ ± SD). The chemistry profiles evaluated included TP, ALB, URE, TBIL, CRE, ALP, ALT, and AST

			UniPron			Post treatment
Test component	Baseline	Placebo (Smugel)	0.4 g	0.8 g	1.2 g	
TP (g/dl)	6.7±1.5	6.1±1.4	6.3±0.9	5.7±0.7	5.7±1.2	6.8±1.2
ALB (g/dl)	4.8±0.7	4.7±0.7	4.9±0.6	4.7±0.9	4.7±0.8	4.6±0.8
URE (mg/dl)	50.7±1.0	49.4±6.4	45.0±6.6	50.0±8.1	49.0±8.5	51±4.5
TBIL (mg/dl)	0.25±0.18	0.27±0.17	0.24±0.15	0.25±0.19	0.26±0.2	0.24±0.17
CRE (mg/dl)	1.1±0.4	0.9±0.2	1.1±0.5	1.0±0.2	1.1±0.1	0.9±0.4
ALP (U/L)	254.0±106.8	204.4±53.9	263.3±111.4	239.6±90.1	254.9±105.6	235.1±119.1
ALT (U/L)	31.2±10.7	27.2±10.0	30.7±8.1	33.1±7.6	32.1±8.9	32.8±4.1
AST (U/L)	43.3±23.6	34.8±7.5	36.9±7.6	34.5±7.5	33.5±6.5	36.3±8.4

3.3. Vaginal Microbiology

A total of 20 sexually mature cycling female baboons were used in this study. Diverse species of both Gram positive, Gram negative bacteria and yeast cells/*Candida* were isolated from the vaginal swabs collected from the animals. Of the Gram positive rods, eight species of *Lactobacilli*, two Coryneform and five other species were isolated. Four species of *Lactobacilli*, namely *L. acidophilus, L. brevis, L. fermentum and L. rhamnosus* were found to be frequent. The proportion of swab results with *L. acidophilus*, for example, were reported as follows; baseline (27%; 17/100); Smugel (23%; 9/40); UniPron 0.4 gm (20%; 8/40); UniPron 0.8 gm 25%; 10/40); UniPron (1.2%; 23/40). Of the coryneform bacteria, both *C. glucuronolyticum* and *C. renale* group were found to be common in the baboon vagina with baseline data having more than 50% of the swabs with both species of bacteria. The proportion of swab with these bacteria was found to be high across the four gel groups (Table 3a).

Table 3a. Percentage frequency isolation of gram positive rods at baseline and during treatment with Smugel (placebo) or UniPron

	% isolation				
			UniPron gel		
Gram positive rods	Baseline	Smugel	0.4g	0.8g	1.2g
Lactobacilli species	N=20	N=5	N=5	N=5	N=5
L acidophilus	27	23	20	15	23
L. brevis	12	18	10	10	15
L. crispatus	4	0	5	5	8
L. delbrueckii ssp delbrueckii	4	3	8	0	5
L. fermentum	21	15	13	18	20
L. pentosus	16	28	30	23	30
L. plantarum	8	0	0	8	10
L. rhanmosus	13	18	15	20	13
Coryneform					
Corynebacterium glucuronolyticum	69	40	43	45	33
Corynebacterium renale group	54	35	33	40	25
Other gram positive rods					
Leuconostoc lactis	19	15	13	8	15
L. mesenteroide ssp mesenteroides	3	3	3	3	3
Bacillus species	16	23	13	18	15
Clostridium species	11	8	3	4	33

Table 3b. Percentage frequency isolation of gram positive cocci at baseline and during treatment with Smugel (placebo) or UniPron

	% isolation				
			UniPron gel		
Gram positive cocci	Baseline	Smugel	0.4 gm	0.8 gm	1.2 gm
Staphylococci species	N=20	N=5	N=5	N=5	N=5
S. aureus	46	38	45	33	38
S. xylosus	14	10	18	15	18
S. hyicus	2	3	8	13	5
S. chromogenes	3	0	0	0	3
S. hominis	3	0	3	0	3
S. lentus	0	0	0	3	3
Streptococcus species					
Aerococcus viridans	71	58	53	55	63
Enterococcus faecalis	13	10	18	18	15
Others					
Lactococcus rafinolactis	21	0	10	5	5

Table 3c. Percentage frequency isolation of gram negative rods and yeast cells at baseline and during treatment with Smugel (placebo) or UniPron

	% isolation				
			UniPron gel		
Gram negative rods & Candida species	Baseline	Smugel	0.4 gm	0.8 gm	1.2 gm
Gram negative rods	N=20	N=5	N=5	N=5	N=5
Escherichia coli	15	20	10	5	18
Gardnerella vaginalis	2	3	3	3	0
Klyuvera spp	0	3	3	3	0
Candida species					
C. albicans	16	8	20	15	15
C. crusei	2	0	0	8	5
C. guilliermondii	4	5	3	0	0
C. tropicalis	0	5	3	3	0

In the Gram positive cocci group, six species of *Staphylococci* and two *Streptococci* were isolated. The Staphylococci included *S. aureus, S. xylosus, S. hyicus, S. chromogenes, S. hominis and S. lentus. S. aureus* was found to be the most common staphylococcus species; baseline (46%; 46/100); Smugel (38%; 15/40), UniPron 0.4 gm (45%; 18/40), UniPron 0.8 gm (33%; 13/40). UniPron 1.2 gm (38%; 15/40). *A. viridans* and *Enterococcus faecalis* were the only *Streptococci* species isolated. *A. viridans* was the most predominant bacteria and was found in more than 50% of the swabs both at baseline and across the four gel groups (Table 3b). Also isolated were four species of Gram negative rods namely *E. faecalis, E. coli, G. vaginalis and Klyuvera* spp. *E. faecalis* and *E. coli* were more common than the other rods in this group (Table 3c). In addition to bacteria, the swabs were also found to harbor four species of *Candida*; *C. albicans, C. krusei, C. guilliermondii and C. tropicalis. C. albicans* was found to be the most common candida in the baboon vaginal swabs collected (Table 3c). A few microbes such as *S. lentus, Kliyuvera* spp and *C. tropicalis* were not reported at baseline, but later appeared from a few swabs collected during treatment with the study gels. Similarly, a few other microbes including *L. rafinolactis, S. chromogenes, G. vaginalis and C. krusei* were reported at baseline, but did not appear during treatment at certain gel arms. Other than the few missing isolates, the proportion of vaginal swabs with microbes was approximately equally distributed both at baseline and across the four treatment groups (Table 3a, Table 3b, and Table 3c).

3.4. Biopsies

There was no detectable incidence of abnormal pelvic examination finding after repeated exposure to three concentrations of UniPron or placebo gels. Evaluation of histological sections by light microscopy indicated that, application of UniPron 0.4 gm, 0.8 gm and 1.2 gm twice weekly did not cause any detectable alterations in the cervicovaginal lining of the baboon vagina as no incidence of abnormal pelvic examination finding was observed. The epithelial architecture of vagina of the baboons treated with UniPron across gel arms was largely comparable to baseline and placebo treated biopsies with no detectable alterations such as erythema, edema and/or erosion of the cervicovaginal epithelial lining and leucocyte infiltration being observed (Figure 1 and Figure 2).

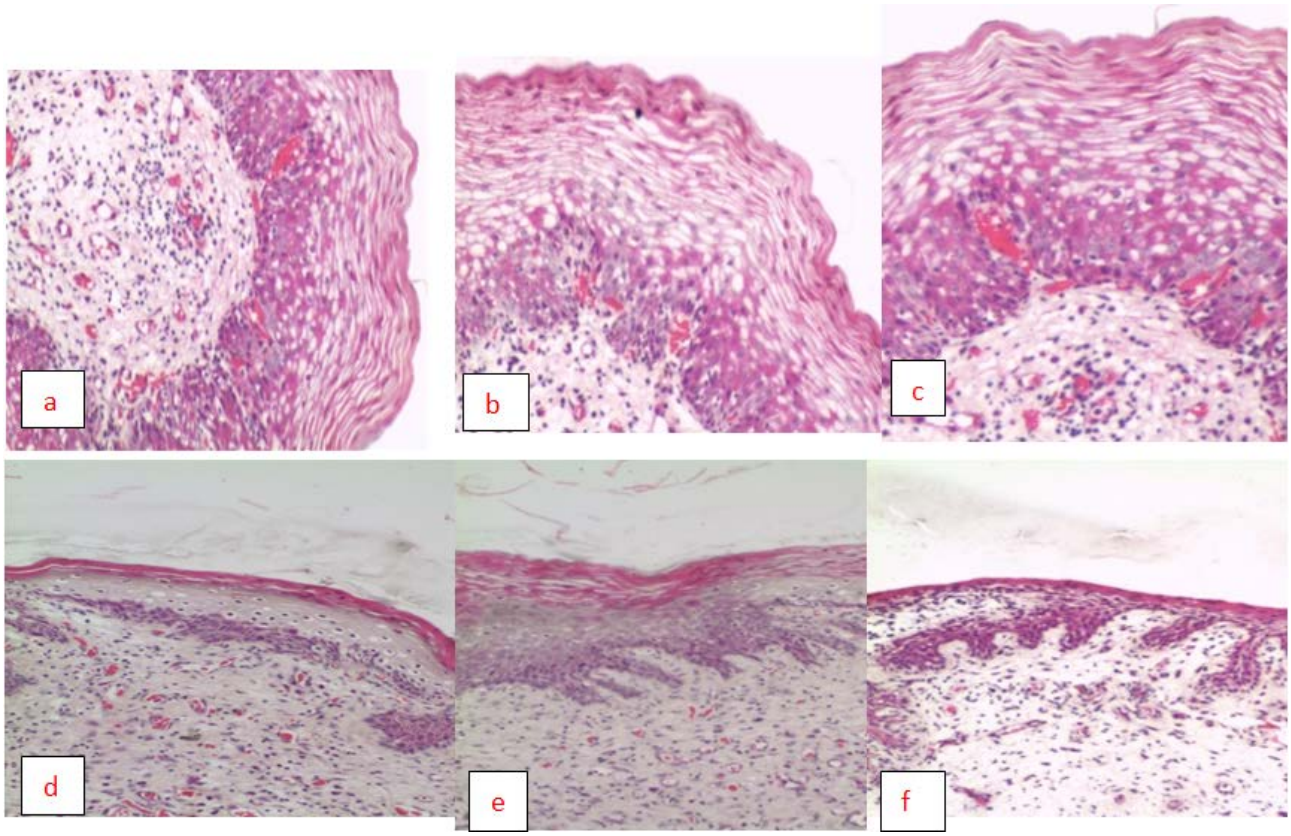

Figure 1a-f. Histology of the vaginal mucosa of baboons at baseline and after treatment with Smugel and UniPron. Vaginal biopsies were obtained from the animals in the follicular and luteal phases of the menstrual cycle. (a) Baseline follicular; (b) Smugel treated follicular; (c) UniPron 0.4gm treated follicular; (d) Baseline luteal; (e) UniPron 0.8gm treated luteal; (f) UniPron 1.2gm treated luteal (H&E ×400)

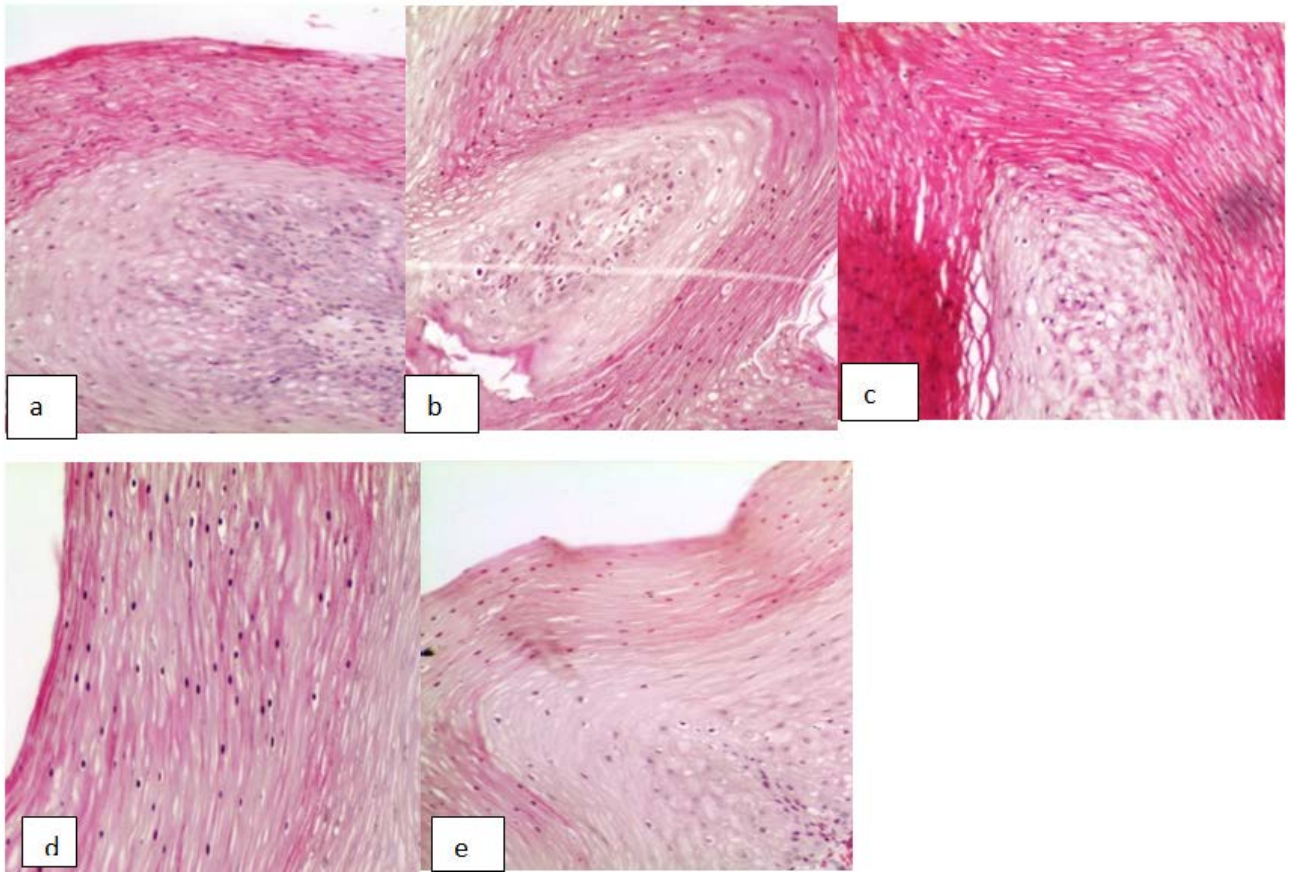

Figure 2a-e. Histology of the cervical mucosa of baboons during luteal phase of the menstrual cycle. (a) Baseline; (b) Smugel treated; (c) UniPron 0.4 gm; (d) UniPron 0.8 gm treated; (e) UniPron 1.2 gm treated (H&E ×400)

4. Discussion

There is renewed emphasis on the development of multifunctional prevention technologies, that is, products designed to address multiple STIs. Dual-protection contraceptive microbicidal products are being designed to prevent STIs and pregnancy [2]. Since consistent and correct use of these products will be critical to their effectiveness, the active pharmaceutical ingredients must be delivered in acceptable vaginal dosage forms such as gels. This study evaluated the safety of UniPron 0.4 gm, 0.8 gm and 1.2 gm compared to placebo administered twice weekly for eight weeks in the baboon model. The baseline data were collected before administration of any intravaginal product to provide for comparisons in the subsequent topical product testing. Repeated exposure of the baboon vagina to the three concentrations of UniPron did not induce detectable toxicity. Assessment of product safety included vaginal pH, clinical chemistry profile, vaginal microflora and cervicovaginal mucosal integrity. The baseline vaginal pH observed was 5.2±0.8 and no significant differences in the pH ($P>0.05$) was found to have occurred during treatment across the four gel arms and one week post treatment (Table 1). Similarly, analysis of blood chemistry parameters revealed no significant differences between baseline and the data obtained during treatment and two weeks post treatment with respect to TP, ALB, CRE, AST, ALT, ALP and TBIL (Table 2) that relate to liver and kidney functions. These findings indicate that the gel neither interfered with baboon vaginal pH, nor clinical chemistry profiles of the parameters tested.

Like the human vagina, the baboon vagina is a dynamic and complicated environment composed of varying microbiological species in variable quantities and proportions. Elucidating how a vaginal product interacts with the vaginal microenvironment constitutes a critical step in evaluating their safety, as the disturbance of this micro environment has been linked with several disease states [20,21] and associated with increased susceptibility to STIs possibly due to related changes in innate defense responses from the epithelial cells [22]. UniPron gel usage did not alter the baboon vaginal microbial flora within the confines of the current study design. However, there were slight variations in the percentage frequency isolation of all the microbes isolated (Table 3a, Table 3b, Table 3c). The differences were not significant and could not be conclusively linked to the use of any of the study gels. For instance, the percentage frequency isolation was high for some microbes such as *C. glucuronolyticum*, *C. renale group* and *A. virudance* (Table 3a, Table3b) both at baseline and across the four gel arms, with *A. viridans* being isolated from more than 50% of the vaginal swabs from each group. For other microbes such as *S. chromogenes*, *S. lentus*, *Klyuvera* spp, *G. vaginalis* and all species of *Candida* except *C. albicans*, (Table 3b, Table3c) the percentage frequency isolation was less than 10% both at baseline and across the four gel arms. The diverse composition of the baboon vaginal microbiota and the variation in percentage frequency isolation could not be attributed to treatment with any of the study gels, but could be as a result multiple factors such as host-specific relationships, composition of vaginal secretions and receptors on vaginal epithelial cell surfaces or vaginal tract tolerance to a variety of different strains of bacteria [23], a feature that might contribute to survival of primate species. In addition the vaginal flora characteristic is also of transient nature.

The healthy cervicovaginal mucosa represents an efficient barrier against STIs and dissemination of pathogens [24]. Histopathology results of biopsies obtained from UniPron treated animals showed that UniPron concentrations of 0.4 gm, 0.8 gm and 1.2 gm were non-toxic and did alter the integrity of the cervicovaginal mucosa (Figure 1 and Figure 2). Abnormal gross pelvic examination finding such as edema, erythrema excoriations or abrations were not identified. Several vaginal products, microbicidal or microbicidal contraceptive trials have been terminated due to safety concerns [16,17]. Vaginal contraceptive products have been available for many years and usually contain the membrane surfactant N-9 as one of the main ingredient. This has been associated with cytotoxic effect on the vaginal cells and increased genital tract inflammation thereby potentiating the transmission of infectiousness of STIs including HIV by recruitment of white blood cells to the genital tract and possibly, by upregulation of genital cytokines [25]. In addition, N-9 is also known to inactivate lactobacilli, which form the normal flora in vaginal tissues [26]. It is essential that the evaluation of the potentially harmful effects of these compounds on the vaginal epithelium and/or normal vaginal flora should be made before these products are used in human clinical trials. Partly due to the recent failures in human microbicide trials [27] there is an emerging consensus that pre-clinical safety and efficacy testing of vaginal microbicidal contraceptive candidates in nonhuman primates should precede to human trials. The development of different dosage forms will help ensure that women can find a method to protect themselves from pregnancy, and potential STIs [28]. Evaluation of the vaginal pH, microbiologic and histology of the baboon model has characterized the baboon vaginal environment's response to repeated topical product application in the absence of the exogenous factors of intercourse and potential infectious ejaculate. The three concentrations of UniPron appeared to be safe and well tolerated based on the results of the study in the baboon model. These data support the need for future studies of this product and demonstrate that it is feasible to use the baboon model for assessment of potential vaginal microbicidal products.

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