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RECENT AND PRODUCTIVE VAGINAL IRRITATION TESTS FOR MICROBICIDAL BIOADHESIVE GELS IN NEW ZEALAND STAT FEMALE RABBITS

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ABSTRACT

Rabbits are highly sensitive to vaginal irritant than human. International Organization for Standardization (ISO) protocol 10993-10 was used, for *in vivo* Rabbit Vaginal Irritation (RVI) test. Twelve healthy, non pregnant, non lactating New Zealand stat female rabbits were taken and four groups of equal animals were made. 1ml of blank and test samples (1% Microbicides (Abacavir sulfate, didanosine carbonate, and tenofovir disoproxil fumarate) loaded nanoparticles of chitosan-polyethylene oxide-polypropylene oxide dispersed 1% HPMC intra vaginal gel formulations) were tested for twice a day for three days on external genitalia, and intra-vaginal tissues. Fourth day was wash out period, and same procedure was repeated for carbopol gel. All animals were evaluated for epithelial ulceration, vascular congestion, swelling, edema, redness, erythrema, vaginal tolerance, and safety of formulations. All rabbits showed no signs of swelling, redness, erythrema, edema, epithelial ulceration, and vascular congestion of vaginal tissues safety of the developed formulations. These Studies were carried out at PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, India.

KEYWORDS: Gel, irritation, microbicide, nanoparticle, rabbit, vagina.

A. INTRODUCTION

Rabbits are highly sensitive to vaginal irritant than human. Some of the shortcomings of the RVI test may be due to the structural differences between human and rabbit vaginal tissue

(Figure 1, 2, and Table no.1). Two-thirds of the rabbit vagina is lined by columnar epithelium (Figure 2), which is structurally distinct from the stratified squamous epithelium (8–12 cells thick) of the human vagina (Figure 1), and is also highly sensitive to vaginal irritants when compared to its human counterpart [19].

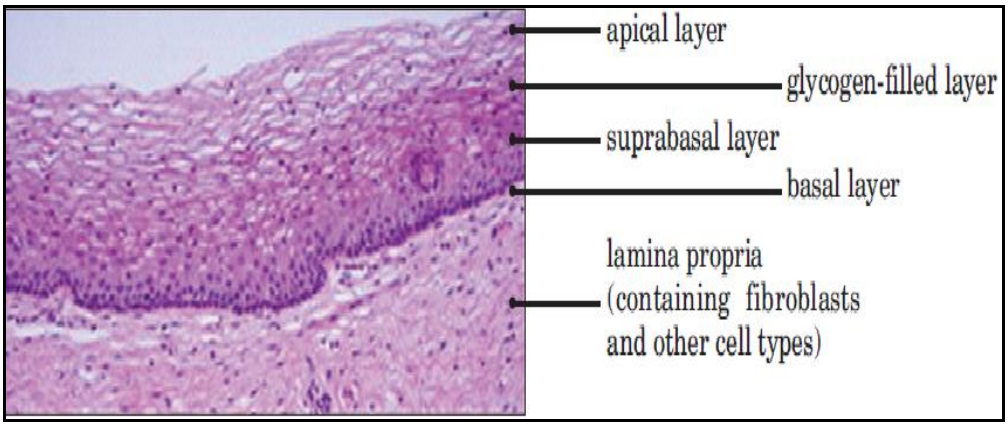

Fig 1: Histology of the vaginal epithelium Human

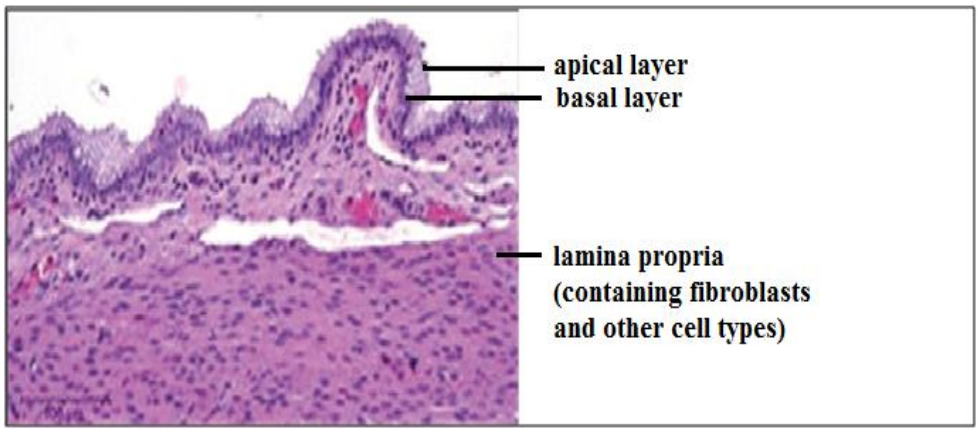

Fig 2: Histology of the vaginal epithelium Rabbit

However, despite the dissimilarities between rabbit and human vaginal tissues detailed above, the rabbit method is preferred and is widely used to determine the vaginal tolerance or mildness of topical microbicidal preparations, because it is a brief and economical test. The *in vivo* Rabbit Vaginal Irritation (RVI) test has long been the preferred choice for vaginal irritation studies [19]. Briefly, the RVI test is performed as follows, 1ml of test material is applied twice daily, for 3 days, through a lubricated catheter, or tuberculin syringe, on the vagina of each of three mature rabbits; the external genitalia are observed daily for any signs of erythema, odema or discharge as a reaction to the exposure to the test materials. After specific time points (chosen to fit the objectives of the study), each rabbit is observed the vaginal tissue. Usually, parts of the cervicovaginal, mid-vagina, and uro-vagina of each

animal are. Each of the three regions of the vagina is scored for epithelial ulceration, leukocyte infiltration, edema and vascular congestion [19]. An overall *individual* irritation score is assigned to each of the three regions, based on a semi-quantitative scoring system which takes into account the endpoints mentioned below [19], as follows: individual score 0 =no irritation; 1 = minimal; 2 = mild; 3 = moderate; and 4 = intense irritation. The scores for each region are combined, and the *total* irritation score is then related to human irritation potential as follows: scores of 0–8 are acceptable, scores of 9–10 indicate borderline irritation potential, and scores of 11 and above are indicative of significant irritation potential (Table 1). The International Organization for Standardization (ISO) protocol 10993-10 is also used, which is based on the treatment of three rabbits for three days.

Table 1: Scoring systems for the assessment of human vaginal irritation in rabbit studies

Species	Endpoints and scoring	Ref.	Comments
Rabbit	**Endpoints:** Epithelial ulceration, leukocyte infiltration, odema and vascular congestion **Individual irritation scoring:** 0 = no irritation 1 = minimal irritation 2 = mild irritation 3 = moderate irritation 4 = intense irritation	[19]	**Test duration:** 7 days The **total irritation scoring** system correlates to human irritation potential as follows: Scores of 0–8 are acceptable Scores of 9–10 indicate borderline irritation potential Scores of 11 and above are indicative of significant irritation potential

Microbicides (Abacavir sulfate, didanosine carbonate, and tenofovir disoproxil fumarate) loaded nanoparticles of chitosan-polyethylene oxide-polypropylene oxide dispersed HPMC/Carbopol intra vaginal gel formulations were tested on external genitalia, and intra vaginal tissues of non pregnant, non lactating New Zealand Stat female rabbits. All animals were evaluated for epithelial ulceration, vascular congestion, swelling, edema, redness, erythrema, vaginal tolerance, and safety of formulations. These Studies were carried out at PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, India.

B. MATERIALS AND METHODS

External genitalia, Intra-vaginal tissue irritation tests on New Zealand stat Female Rabbits.

(International Organization for Standardization (ISO) Protocol 10993-10 Guidelines)

Study design.

Animals used: Twelve healthy, non-pregnant, non-lactating New Zealand Stat female rabbits

Weight range: 1800 – 2000 gm

Samples **:** 1% C-PEO-PPO-1% HPMC gel formulation-Placebo

: 1% C-PEO-PPO_A1% HPMC gel formulation

: 1% C-PEO-PPO_D1% HPMC gel formulation

: 1% C-PEO-PPO_T1% HPMC gel formulation

: 1% C-PEO-PPO 1% Carbopol gel formulation-Placebo

: 1% C-PEO-PPO_A1% Carbopol gel formulation

: 1% C-PEO-PPO_D1% Carbopol gel formulation

: 1% C-PEO-PPO_T1% Carbopol gel formulation

Dose **:** 1ml

Study duration **:** Seven days.

Animal housing and feeding conditions

For feeding, normal diet was used with an unrestricted supply of drinking water.

Procedure

Twelve healthy, non pregnant, non lactating New Zealand stat female rabbits weighing between 1800-2000 gm were taken and four groups of equal animals were made. 1ml test samples were applied with the help of cotton swab on external genitalia of vaginal tissues, and with syringe on intra vaginal tissues as follows, First group was negative control and treated with placebo (1% C-PEO-PPO 1% HPMC gel formulations second group with 1% C-PEO-PPO_A1% HPMC, third group with 1% C-PEO-PPO_D1% HPMC and fourth group with 1% C-PEO-PPO_T 1% HPMC gel formulations for once a day for three days, and observed for any kinds of irritations, swelling, redness, vaginal discharge. Fourth day was wash out period, and same procedure was repeated on same groups of animals for 1% C-PEO-PPO1% Carbopol, 1% C-PEO-PPO_A 1% Carbopol, 1% C-PEO-PPO_D 1% Carbopol 1% C-PEO-PPO_T 1% Carbopol gel formulations respectively.

Table 2: *Irritation* test performed external vaginal genitalia and Intra-vaginal tissues for HPMC/Carbopol gels.

Rabbit No.	1		2		3		4	
Time Point	**10.00am**	**6.00pm**	**10.00am**	**6.00pm**	**10.00am**	**6.00pm**	**10.00am**	**6.00pm**
Sample applied	1% C-PEO-PPO 1%HPMC		1% C-PEO-PPO_A1%HPMC		1% C-PEO-PPO_D1%HPMC		1% C-PEO-PPO_T1%HPMC	
Dose	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
1st day	▲■	▲■	▲■	▲■	▲■	▲■	▲■	▲■
2nd day	▲■	▲■	▲■	▲■	▲■	▲■	▲■	▲■
3rd day	▲■	▲■	▲■	▲■	▲■	▲■	▲■	▲■
4th day	**Wash out period**							
Sample applied	1% C-PEO-PPO 1%Carbopol		1% C-PEO-PPO_A1%Carbopol		1% C-PEO-PO_D1%Carbopol		C-PEO-PPO_T1%Carbopol	
Dose	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
5th day	▲■	▲■	▲■	▲■	▲■	▲■	▲■	▲■
6th day	▲■	▲■	▲■	▲■	▲■	▲■	▲■	▲■
7th day	▲■	▲■	▲■	▲■	▲■	▲■	▲■	▲■

***C**-Chitosan,* ***PEO**-Poly ethylene oxide,* ***PPO**- Poly propylene oxide,* ***HPMC**- Hydroxy Propyl Methyl Cellulose,*
***A**-Abacavir sulfate,* ***D**- Didanosine carbonate,* ***T**-Tenofovir disoproxil fumarate,* ▲*-Applied in vagina,* ■*-Applied intra-vaginal tissue*

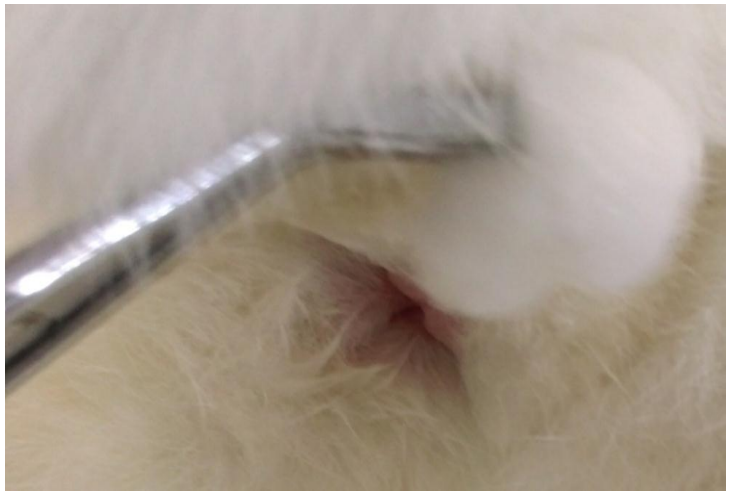

Fig 3: Application of C-PEO-PPO_A-HPMC gel formulation on vaginal genitalia New Zealand stat female rabbit no-5

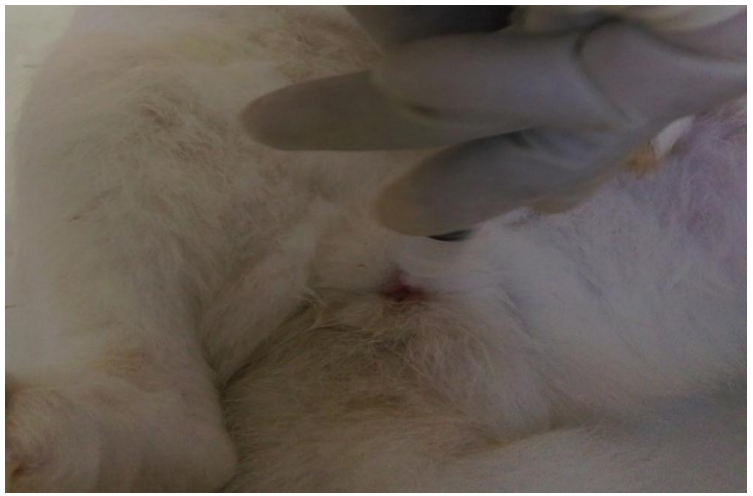

Fig 4: Application of C-PEO-PPO_A-Carbopol gel formulation External on Intra-vaginal tissues of New Zealand stat female rabbit no-5

C. RESULTS AND DISCUSSION

Table 3: Observation/*Irritation* scores of external vaginal genitalia, intra-vaginal irritation test for HPMC/Carbopol gels.

Rabbit No.	1				2				3				4			
Sample applied	1% C-PEO-PPO 1%HPMC				% C-PEO-PPO_A 1%HPMC				1% C-PEO-PPO_D 1%HPMC				1% C-PEO-PPO_T 1%HPMC			
Time Point	10.00am		6.00pm		10.00am		6.00pm		10.00am		6.00pm		10.00am		6.00pm	
Dose	1ml		1ml		1ml		1ml		1ml		1ml		1ml		1ml	
Observation / Irritation scores	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score
1st day	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0
2nd day	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0
3rd day	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0
4th day	**Wash out period**															
Sample applied	1% C-PEO-PPO 1%Carbopol				%C-PEO-PPO_A 1%Carbopol				1%C-PEO-PPO_D1%Carbopol				1%C-PEO-PPO_T1%Carbopol			
Dose	1ml		1ml		1ml		1ml		1ml		1ml		1ml		1ml	
Observation / Irritation scores	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score	Obs.	Score
5th day	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0
6th day	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0
7th day	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0	NS, NR, NO,NVC	0

***C**-Chitosan, **PEO**-Poly ethylene oxide, **PPO**-Poly propylene oxide, **HPMC**- Hydroxy Propyl Methyl Cellulose, **A**-Abacavir sulfate, **D**-Didanosine carbonate, **T**-Tenofovir disoproxil fumarate, **N**-No, **S**-Swelling **R**-Reddening, **O**-Odema, **VC**-Vascular congestion. **0**- no irritation, **1**-minimal irritation, **2**-mild irritation, **3**-moderate irritation, **4**-intense irritation*

1ml C-PEO-PPO_A-HPMC gel formulation applied with cotton swab on external vaginal genitalia of New Zealand stat female rabbit no-5 (Fig 3) and 1ml C-PEO-PPO_A-Carbopol gel formulation applied with the help of syringe on Intra vaginal tissues of New Zealand stat female rabbit no-5 (Fig 4) showed no signs of swelling, redness, erythrema, edema, epithelial ulceration, and vascular congestion of vaginal tissues of New Zealand Stat female rabbit no-5 (Fig 5) so irritation scoring was zero (0), while all rabbits showed no signs of swelling, irritation, redness, vaginal discharge to HPMC/Carbopol gel formulations.

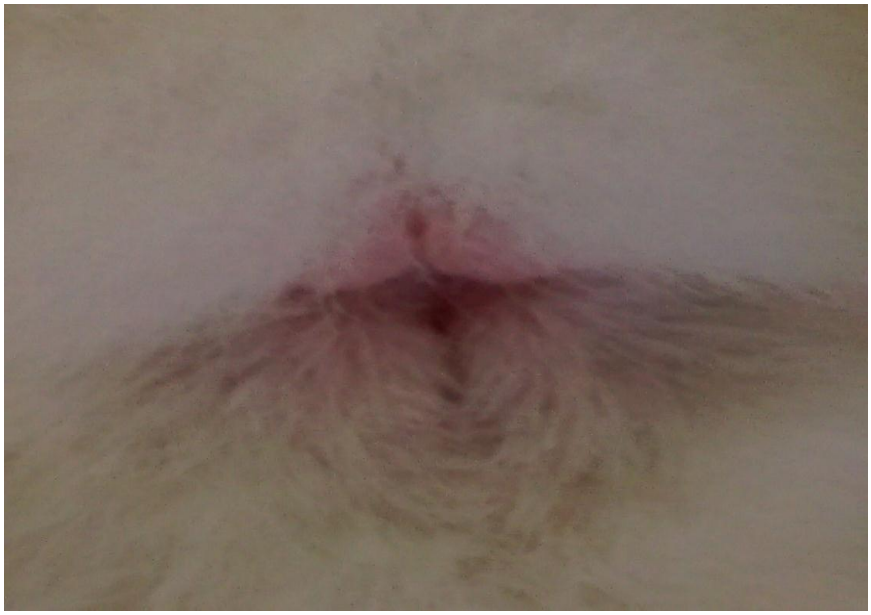

Fig 5: No swelling, *irritation* on vaginal tissues of New Zealand Stat female rabbit no-5 due to HPMC/Carbopol gel formulations

D.CONCLUSION

Rabbit vaginal irritation tests showed safety of the developed formulations. However, further research needs to be directed w.r.t. detailed large scale animal studies are necessary to assess the potential of these intra vaginal gels.

E. ACKNOWLEDGMENT

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