



A REVIEW ON RABBITS VAGINAL IRRITATION (RVI) TEST OF BIOSTIMULANT FORMULATION

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Abstract: Mucous membrane act as a natural protective barrier to any disease and when we use some vaginal formulations frequently it causes damage in the epithelium. The vaginal mucous membrane of rabbit was same as human in compare to rat due to presence of a cornified epithelial cells which is not present in rat therefore the vaginal mucous membrane of rabbit have greater sensitivity towards vaginal irritants. Rabbit have a simple cuboidal or columnar epithelium for this reason, vaginal irritation test frequently performed in rabbit in this review we can see the effect of biostimulant formulation on rabbit vagina after the experiment we found that there is no any sign of oedema and edema this results shows the X drug do not have any irritant effect [1].

Index Terms - Mucous membrane, Epithelium, vaginal irritants, Biostimulant formulation Oedema, Edema

1. OBJECTIVE

To determine the vaginal tolerance and mildness of Biostimulant formulation on vaginal tissues of rabbits through mucous membrane irritation test.

2. INTRODUCTION

The rabbit vaginal area appears to be an appropriate location for evaluating the efficiency of chemicals whose action provide local effect and drug delivery through vagina is a method of systemic drug delivery it means drugs directly reaches into the blood due to its wide surface area, abundant blood supply and lack of the first pass effect[2]. Previous research shows that the vagina of rabbit is a muscolumembranous cylindrical duct which is about 13 to 14 cm long. The 2/3 upper part of vagina forms a 'cervico vaginal canal' and this portion is similar as endocervix of female and after this study the rabbit was helpful as a experimental model for various scientific studies[3] Various previous studies shows that how the grafted heterologues porcine bone heals the rabbit bone in cancellous bone defect of rabbit so it means porcine derived bone graft material also have osteoconductive properties and other materials like putty and gel-40 displays undesirable effects. [4] Several studies shows that the vagina is the easy targets for oestrogen and progesterone and both these levels are significantly raised after menopause.[5]

Anna Michelitsch and his co-workers reported that Rabbit is the third most commonly used tiny model animal. This species is mostly used for immunization, but also employed for implant research and it is used as a model for a variety of human infectious disorder such as infection with noro virus, syphilis, tuberculosis or human papilloma virus. Rabbit have previously been shown to be sensitive to MERS-CoV then in silico studies utilizing diverse animal ACE2 receptors show that rabbits were expected to be susceptible to SARS-CoV-2 furthermore, it was demonstrated that SARS-CoV-2 replicated in the rhesus monkey. Intranasally inoculating three-month-old New Zealand white rabbits shows the virus replication and shedding from the nose and throat followed by seroconversion. The olfactory epithelium displayed hyperplasia and hypertrophy, as well as multifocal eosinophilic and lymphoplasmacytic infiltration, after four days of infection. In an immunohistochemical staining test of the lungs there is no any antigen was found and the neutrophills and alveolar macrophages amount were increased. On the other hand mildly thickened septa and inflammation in the cells were founded in the bronchiole's terminal region. After the study was completed the researchers was seen mild necrosis in the alveolar epithelial cells, as well as per bronchiolar lymphoid tissues have proliferation and this proliferation causes enlargement of the lymph nodes after the conclusion, clinical illness was not seen in rabbits, including histopathological abnormalities.[6]

According to some studies Rabbits phylogenetically related to the humans in compare to rodents and belonging to Leporidae family and lagomorpha order. They are calm and simple to handle at animal house facilities and are of a manageable size. Various

rabbit strains exist, including Dutch-belted and Himalayan. Due to less health concerns, New Zealand white rabbits are the most widely utilized laboratory breed. The rabbits show structural similarity with human in case of cardiovascular system so that they can be used in cardiovascular research. Their broad benefits, such as a similarity in eye size and bone metabolism, as well as a unique aspect of lipoprotein metabolism they are being used as animal models in more human atherosclerosis related studies, lipid metabolism joint and bone diseases, eye illness. Pneumocystis pulmonary infections have also been studied using SCID rabbit models.[7]

According to many conducted experiments and resident based proof provide information about rabbit adipose-derived mesenchymal stem cells (rAT-MSCs). The researchers used flow cytometry and PCR methods to characterize rAT-MSC, particularly digital droplet PCR, which validated the expression of selected markers at mRNA level. After the experiment result shows that rabbit AT-MSCs and human AT-MSCs are quite comparable. A positive expression of surface and intracellular markers like CD29, CD44, CD49F, CD73, CD90, CD10 etc was confirmed by using both techniques such as immunostaining and PCR method furthermore, the pluripotent marker expression is important which is NANOG, OCT4 and SOX2 that was true. We can conclude from these observations that effective neurodifferentiation was achieved in rMSCs culture, as evidenced by the presence of particular neuronal markers that is ENO2 and MAP2. Finally, more research on rAT-MSC is needed to give greater characterization of all these cells which can be used for various cryopreservation and also for clinical purposes. After the experiment rabbit AT-MSCs cryostored in a gene bank could be a valuable genetic supply for endangered breeds.[8]

Rabbit Vaginal Test (RVI)

The main objective of this test is to estimate the tolerance of epithelial tissue of rabbit vagina and various studies show that this test was generally used for study vaginal toxicity. The RVI test is carried out as: A lubricated catheter, or a tuberculin syringe, is placed daily for 10 days into the vagina of each of three to four mature rabbits and catheter or tuberculin syringe contains 1 mL of test material then we observe external genitalia daily for any evidence of erythema, oedema or any other discharge.

After the experiment, the histopathologist euthanized each rabbit and removed the vaginal tissues for observation. The rabbit vagina has three parts that is cervico-vagina, mid-vagina and uro-vagina and these parts were fixed and then sectioned using any in a paraffin wax tray then stained by using haematoxylin-eosin (H&E). After that each part of vagina scored for leukocyte infiltration, vascular congestion, epithelial ulceration and oedema.[9]

3. MATERIAL AND METHODS

3.1 For Mucous Membrane Irritation Test-

Animal used-

Species-Rabbit

Strain- New Zealand

3.1.1 Material-

- ✓ Animal facility consumables (cages, bottles, racks etc)
- ✓ Glassware

3.1.2 Chemicals and reagents-

- ✓ Test material

3.1.3 Environmental conditions-

- ✓ Room temperature-22±3°C
- ✓ Relative Humidity- 30-70%
- ✓ Housing- UV sterilized standard iron mesh Cage
- ✓ Feed- Rabbit pellet diet Altromin 2123.
- ✓ Water-Deep bore well water filter through Aqua guard purifier.

3.2 Procedure-

- 1) Test material shown to be irritant having pH of less than or equal 2.0 or more than or equal to 11.5 will be excluded from the test.
- 2) Acclimatize healthy young adult females rabbits not less than 2 kg for 5-7 days prior to the test.
- 3) Randomly assign the animals to the treatment groups after acclimatization.
- 4) Three rabbits used to evaluate test item and three for control groups.
- 5) For testing of liquids, a dose of 0.1 ml per animal will be used. The test item will be applied directly in the vagina.
- 6) Solid test material grounded to fine dust and directly applied to vaginal mucosa at the dose rate of 100 mg per animals.
- 7) Record the body weight of rabbit prior to exposure of the test item.
- 8) Apply test material at the upper vault of vagina for 4 hours. At the end of exposure period, remove the residual substance by using distilled water without disturbing venial epidermis.
- 9) Observe animals for sign of erythema, oedema and the responses were scored at 4, 24, 48, 72 hours and 7, 14 and 21 days after exposure.
- 10) Assess the mucous membrane irritation reaction following table of numerical scoring system of Draize et al (1944) at 4, 24, 48, 72 and 21 days after exposure.

Table 1.1 Grading of skin reaction

Erythema and Escher formation	Score	Oedema formation	Score
No erythema	0	No oedema	0
Very slight erythema (rarely perceptible)	1	Very slight oedema (rarely perceptible)	1
Well defined erythema	2	Slight erythema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate oedema (raised approximately 1 mm)	3
Severe erythema (beet redness) to slight Escher formation (injuries in depth)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible score	4	Maximum possible score	4

4. RESULTS & DISCUSSION

4.1 Clinical sign observation-

After the experiment there is no any signs of Oedema and Erythema were observed and which is mentioned in below table

Table 1.2 Vaginal mucous membrane irritation scores of female rabbits

Females		Erythema(score)							Oedema(score)						
Group	Animal no.	4h rs	24h rs	48h rs	72h rs	7 days	14 days	21d ay	4 hrs	24 hrs	48 hrs	72 hrs	7 days	14 days	21 days
Control	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean score		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Treatment	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean score		0	0	0	0	0	0	0	0	0	0	0	0	0	0

5. CONCLUSION

The main goal of this experiment was to examine the effect of test substance on the vaginal mucous membrane of rabbit In present study we found that there is no oedema and erythema formation takes place in rabbit vagina after the application of test substance and after observation the scoring was zero.

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