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Short Communication

Pharmacological study to evaluate acute eye irritation/corrosion activity of Vasanjana - an Ayurvedic formulation

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Intramuscular fat from animal origin can be used for effective and better management of dry eye syndrome. Vasanjana is one of such Ayurvedic ocular preparation for topical use in dry eye of human beings. Present study was undertaken to evaluate the acute eye irritation/corrosion effect of Vasanjana ointment in rabbits as per standard protocol of OECD 405 guideline to develop its safety profile. Vasanjana ointment was applied to one eye of rabbits in a single dose of 100 mg in conjunctival sac of each animal. The animals were observed for 21 days post administration of the test formulation for presence or absence of any irritation/corrosion effect in treated rabbits. The result of present study revealed that no ocular lesions, ulceration, corneal opacity, congestion or chemosis were observed during and after the period of study. Thus it can be concluded that Vasanjana did not possess any acute eye irritation/corrosion effects in rabbits.

Key words: Vasanjana, Dry eye syndrome, irritation, corrosion

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1. INTRODUCTION

The Cultural, social and environmental changes that accompanied the modernization of the society all over the globe have lead to increase in the incidence of certain diseases that includes eye disorders too. Dry eye syndrome is one such disorder of the tear film due to tear deficiency or excessive evaporation, which

causes damage to inter palpebral ocular surface and is associated with symptoms of ocular discomfort.¹ In modern system of medicines, artificial tears, anti-inflammatory drugs if syndrome associated with inflammatory changes and surgical management are practiced for dry eye syndrome. In Ayurvedic system of medicines many ailments prescribed for dry eye syndrome. Vasanjana, is one of them Ayurvedic topical ocular medicine indicated in Shushkakshipaka (i.e. dry eye syndrome) in ancient Ayurvedic classics.²

Vasanjana contains drugs like Vasa (intramuscular fat) from pork meat, Shunthi (*Zingiber officinalis*), Saindhava (rock salt). Vasanjana is claimed to be effective in Shushkakshipaka (Dry eye syndrome) in Ayurvedic classics. Vasa is animal muscle fat and its use in ophthalmology is having history of thousands of years,³ but in clinical parlance it has been almost out of practice of today's ocular therapeutics. One of the many health claims attributed to ginger is its purported ability to decrease inflammation, swelling, and pain.⁴ Dried ginger extract and a dried gingerol-enriched extract were each reported to exhibit analgesic and potent anti-inflammatory effects.⁵ Shogaols appear to be more potent than the gingerols, and most of the compounds cause aversive or nociceptive responses mediated by TRPV1 when applied to the eye or following subcutaneous injection to the hind paw, respectively.⁶ Saindhava may aid in the secretion in the eye hence may be useful for lubricating the dry eye.

Though Ayurvedic classics never document any adverse drug reaction or eye toxicity but no evidence of its safety as a topical medicine could be traced in any classical literature. Hence, it is necessary to evaluate its eye irritation/corrosion properties because today's scientific community needs ocular surface safety profile of drugs for human use for its global acceptance. With this background the present study

was undertaken to evaluate the in vivo acute ocular irritation/corrosive effect of Vasanjana in rabbit as per OECD 405 guideline⁷ for revalidation of safety data.

2. MATERIALS AND METHODS

2.1 Test formulation

Test formulation Vasanjana was prepared in Departmental laboratory of Shalakyatantra, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar, Gujarat, India; as per classical guidelines in aseptic condition. Vasanjana, intramuscular fat extracted from pork meat (by boiling in water) was taken 1 part, 0.5 % *Shunthi* (*Zingiber officinale* Rosc.) powder & 1% *Saindhava* (*Sodii chloridum*) powder were mixed by trituration in Granite mortar pestle. The final preparation was semisolid in consistency (like ointment) so was filled in sterile collapsible aluminium tubes for its easy application in the eye.

2.2 Animals

Healthy and young Albino Rabbits were used for this experimental study. The animals were housed individually. They were exposed to 12 hours light and 12 hours dark cycle with the relative humidity of 50 to 70% and the ambient temperature was $22 \pm 03^{\circ}\text{C}$. All animals were kept on same environmental conditions throughout experimental period. "Amrut" brand feed supplied by Pranav Agro Ltd. was provided throughout the experimental period. Drinking water was given *ad libitum*.

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC/15/2013/Ph.D. 36) in accordance with the guideline formulated by CPCSE, India.

2.3 Dose fixation and schedule

The dose for acute eye irritation/corrosion test was fixed at 100 mg as per maximum dose of OECD 405 guideline for testing of solids. The dose of Vasanjana was placed in the conjunctival sac of one eye of each

animal. The untreated eye of rabbit served as the control.

2.4 Experimental protocols

Before initiating the study test substance was applied on the skin of the animals and observed for 24 hours for erythema, irritation etc. However no such effect was seen in rabbit. In vivo test performed initially by using one animal for test formulation. After confirming the non-corrosive or non-irritant nature of Vasanjana, the negative response was confirmed by using two additional animals. Total three rabbits were used for this experimental study on Vasanjana ointment.

Sixty minutes prior to test substance application (TSA-Vasanjana), buprenorphine 0.01mg/kg was administered by subcutaneous injection (SC) to provide a therapeutic level of systemic analgesia. Five minutes prior to Vasanjana application, topical ocular anaesthetic (0.5% proparacaine) was applied to both eyes. The test formulation at the dose of 100mg was placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lid was then gently held together for about one second in order to prevent loss of the material. The eye of each animal that was not treated with a test article but which was treated with topical anaesthetics, served as a control.

Eight hours after Vasanjana application, meloxicam 0.5mg/kg SC was administered to provide a continued therapeutic level of systemic analgesia. The animals were evaluated for the entire duration of study for clinical signs of pain, distress e.g. repeated pawing or rubbing of the eye, excessive blinking, and excessive tearing. The animals were observed several times on first three days for any effect of test formulation then after at least twice daily with minimum of 6 hrs between observations for clinical sign of pain, distress etc. The animals were observed for 21 days post administration of the test formulation.

2.5 Clinical observations and grading of eye reactions

The treated and control eyes of rabbits were evaluated by means of slit-lamp bio microscope for the presence or absence of ocular lesions post Vasanjana application. Any other lesions in the eye (vascularisation, pannus formation, adhesions, staining and anterior chamber changes) or adverse systemic effects were reported.

The grades of ocular reaction (conjunctivae, cornea and iris) were obtained and recorded following test substance application. Observations were performed and recorded at a 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours, 7 days, 14 days, and 21 days in order to determine the status of the lesions, and their reversibility or irreversibility.

2.6 Grading of ocular lesions

Cornea

- Opacity: degree of density
- No ulceration or opacity 0
- Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible 1
- Easily discernible translucent area; details of iris slightly obscured..... 2
- Nacrous area; no details of iris visible; size of pupil barely discernible.....3
- Opaque cornea; iris not discernible through the opacity..... 4

Iris

- Normal..... 0
- Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect...1
- Haemorrhage, gross destruction, or no reaction to light2

Conjunctivae

- Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
- Normal.....0
- Some blood vessels hyperaemic (injected).....1
- Diffuse, crimson colour; individual vessels not easily discernible.....2

Diffuse beefy red3

Chemosis

Swelling (refers to lids and/or nictating membranes)

Normal.....0

Some swelling above

normal.....1

Obvious swelling, with partial eversion of lids

.....2

Swelling, with lids about half

closed.....3

Swelling, with lids more than half

closed..... 4

3. RESULT AND DISCUSSION

Vasanjana ointment at dose of 100mg did not produce any type of ocular lesions in the treated eye of rabbits at any stage of post Vasanjana application. Adverse systemic effects were not observed in rabbits during experimental periods of 21 days. Vasanjana ointment produced no sign of ocular irritation i.e. redness, lacrimation swelling of conjunctiva and lid chemosis. Treated eyes of rabbits were observed almost normal and there after no any signs of toxicity were seen.

Preparation have semisolid consistency hence retains for longer duration and may not easily and completely removed from eyes by physiological mechanism still it did not produced any sign of irritation/corrosion. Iris of rabbits was normal all the time of observation. Cornea also did not show any ulcerations or opacity. According to Ayurvedic theories regarding body physiology and pathogenesis of diseases whenever dryness is there in any organ or structure, the fats are useful to attenuate it.⁸ Vasanjana is hence an ideal formulation as explained in Ayurvedic literature, that does not possess any corrosive or irritant properties, however it is used in curing loss of body elements and nourishing in nature.⁹

4. CONCLUSION

Form the present study, it is concluded that Vasanjana have no any serious toxic effect on iris, cornea, conjunctiva, lids or any ocular parts of rabbit.

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