www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(9): 394-396

www.thepharmajournal.com
Received: 25-07-2019
Accepted: 28-08-2019

Dr. Asha PN
Ph.D. Scholar, Sree Sankara University of Sanskrit, Kalady, Kerala, India

Correspondence
Dr. Asha PN
Ph.D. Scholar, Sree Sankara University of Sanskrit, Kalady, Kerala, India

Acute dermal irritation study of vishakallu in rats

Dr. Asha PN

Abstract
The black stone is a piece of bone treated to be used against the bites of the snakes, scorpions, leeches, and spider. The method of preparation of black stone is explained like this, first animal thigh bone is collected and wash thoroughly and cut into a small pieces of 5 to 6cm long. Rub with sandpaper, wrap the pieces in aluminum foil, place in a charcoal fire for 15 to 20 minute. When we apply the black stone, wait for the stone to absorb all the poison from the attacked area and it falls down. After that the stone is cleansed by putting it in milk or some herbal medicine, here an attempt to evaluate the acute dermal irritation study of vishakallu in rats.

Keywords: Vishakallu, snake, black stone, kriyakaumudi, floklore

Introduction
Primary skin irritation is the production of irreversible inflammatory changes in the skin following the application of a test substance as it is involves the intraction of a chemicals with the sensory receptors in the skin at the site application.
Objective of study-The objective of study tittled acute dermal irritation study of vishakallu in rats was to assess the irritation profile of test material, vishakallu, when administrated topically on wister albino rats on the basis of this study, the possible hazard likely to arises from exposure of the skin to the test substance can be assessed.
Methods- The methods was designed to meet the requirements of the OECD guidelines for testing of chemicals on conduct the acute dermal irritation.

Test System and Management
Animal Species ; Rats
Strain ; Wister albino rats

Justification for Selection of Species
As per OECD Guideline 404 albino rabbits are the preferred animal species for dermal irritation/corrosion studies. As per the Guidelince document on integrated approaches to testing and assessment for skin corrosion and irritation, in vivo testing in rabbits, should not be undertaken until all available data relevant to the potential dermal Corrosivity/irritation of the chemical, based on any pre-existing test data, have been evaluated1-10. The vishakallu sample have not been scientifically validated for its safety on dermal application till now. Hence a new experiment have to be conducted in an experimental animal model other than rabbits, which is of a lower species grade like Wister Albino Rats.
Source: Care Keralam ltd, Koraty, Thrissur
No: of animal and sex: 3 female nulliparous and non-pregnant rats Body weight range: 200 – 250 g Age at treatment: 8 to 12 weeks Identification: Cage cards

Study Design
Approximately 24 hours before the test, hair on the dorsal area of the trunk of the animals, were removed by close clipping. The test sample, vishakallu was applied in a single dose of 0.5g to the skin of experimental animal, untreated skin areas of the test animal served as the control. The test sample was applied to a small area (approximately 6cm2) of skin and covered with a gauze patch, which was held in place with non-irritating tape. The test sample, vishakallu was first applied to the gauze patch, which is then applied to the skin. The patch was loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. The gauze patch was attached to the skin in such a manner that there was good contact and uniform distribution of the chemical on the skin.

Table 1: Method of standrads to follow in experiments

Conditions	Animals were housed under standard laboratory conditions. Air conditioned with adequate fresh air through IVC system. room temperature 220.0 to 240c,humidity 57-65%, with 12 hr light and 12 hr dark cycle, and all are recorded daily
Housing	Single animal was housed in a Polysulfonate cage (size-L300X B 170X H 140mm) with stainless steel top grill mesh having facilities for holding pelleted food and drinking water. sterilized paddy husk was provided as bedding material
Acclimatization	Period-7 days (veterinary examination of all the animals was recorded on the first and 7th day)
Diet	The animals were fed *ad* libitum. Pellected lab rodent feed was provided
Water	Deep bore- well water passed through activated charcoal filter and exposed to ultraviolet rays in aquaguard water filter cum purifier was provided in plastic water bottles with stainless steel sipper tube.

In the initial test, up to three test patches were applied sequentially to the animal. The first patch was removed after three minutes. A second patch was applied at a different site and removed after one hour and a third patch was applied and removed after four hours, and the response was graded. The results of the initial test was confirmed using two additional animals, each with one patch, for an exposure period of four hours.

At the end of the exposure period, which is normally 4 hours, residual test sample was removed, without altering the existing response or the integrity of the epidermis.

To determine the reversibility of effects, the animals were observed up to 14 days after removal of the patches. All animals were examined for signs of erythema and Oedema, and the responses scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal. For the initial test in one animal, the test site was examined immediately after the patch has been removed.

Table 2: Dermal reactions were graded and recorded according to the grades

Erythema and eschar formation grade	Grade
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to eschar formation preventing grading of erythema	4

Table 3: Edema formation were graded and recorded according to the grades

Edema formation	Grade
No edema	0
Very slight edema (barley perceptible)	1
Slight edema (edges of area well raised)	2
Moderate edema(raised approx.1mm)	3
Severe edema (raised more than 1mm and extending 4 beyond area of exposure)	4

Safety precautions

Gloves, cap and face mask were used in addition to protective body garments used and rubber slipper to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test items

Observation

Skin reaction at the site of application was subjectively assessed and scored once daily at 1,24,48,72 hours, 7 and 14 days after patch removal (post –test observation period) accordingly

Result

The computer printout of the data were verified against raw data. All findings were presented in the report as per the standard reporting procedure.

Table 4: Evaluation of Primary Skin Irritation

Evaluation	Score
Non irritant	0.0
Negligible irritant	0.1 - 0.4
Slight irritant	0.41-1.9
Moderate irritant	2.0-4.9
Severe irritant	5.0-8.0

No dermal irritation was observed. Treated skin of all three rats appeared normal throughout the observation period.

Conclusion

The primary skin irritation index of the test material was calculated and was found to be 0.00. Hence, it was concluded that the test substance, vishakallu was non- irritant to the rat skin.

Reference

1. Draize JH, Woodward G, Calvery OH. Methods for the study of irritation and toxicity of substance applied topically to the skin and mucous membrane. J Pharm. Exp, Ther. 1994; 82(3):377-390.
2. Draize JH. Dermal and eye toxicity tests In: Principles and procedure for evaluating the toxicity of household substance, National Academy of Sciences, Washington DC, 1977, 48-49.
3. James O, Sunday AB. Evaluation of acute drmal irritation and wound contraction by *Gymnema sylvestre* and *Datura metel* Extracts in rats. American journal of Biomedical and life sciences. 2014; 2(4):83-88.
4. Krishnaraju AV. Sundararaju D, Vamshikrishnan U, Suryachandra R, Machiraju G, Sengupta K *et al*. Safety and toxicological evaluation of Aflapin R: A Novel boswella-derived anti- inflammatory product. Toxicology mechanisms and methods. 2010; 20(9):556-563.
5. Organization for Economic Co-operation and Development (OECD).OECD Guidelines for testing of chemicals, section 4: Health Effect, test No. 404: Acute dermal irritation/corrosion. OECD publishing: paris, france. 2002; 2:1/13-13/13.
6. Pichayakoran W, Suksaeree J, Boonme P, Taweepreda W, Ritthidej GC. Prepration of deproteinized natural rubber latex and properties of filims formed by itself and several adhesive polymer blends, industrial and engineering chemistry research. 2012; 51:13393-13404.
7. Sekizawa J, Yasuhara K, Suyama Y, Yamanaka S, Tobe M, Nishimura M. A simple methods for screening assessment of skin and eye irritation, J Toxicol sci. 1994; 19:25-35.
8. Soujanya C, lakshmi satya B, Lokesh reddy M, Manogna K, Ravi Prakash P, Ramesh A. Formulation and in vitro and in vivo evaluation of transdermal patches of lornoxicam using natural permeation Enchancers, Int J Pharm Pharm Sci. 2014; 6(4):282-286.

9. Suksaree J, Boonme P, Taweepreda W, Ritthidej GC, Pichayakoran W. Characterization, *in vitro* release and permeation studies of nicotine transdermal patches prepared from Deproteinized Natural Rubberlatex Blends. Chem. Eng. Resw. Des. 2012; 90(7):906-914.
10. Teshome K, Gebre-Marian T, Asres K, Engidawork E. Toxicity studies on dermal application of plant extract of dodonaea viscose used in Ethiopian traditional medicine, Phytotherapy research. 2010; 24:60-69.