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A study on the acute dermal irritation of a novel polyherbal anesthetic formulation for parenteral administration in wistar albino rats

Abstract

The major aim of the present study is to assess the acute dermal irritation of a novel topical polyherbal anaesthetic formulation for parenteral application. Currently, this formulation does not have any scientific evidence on the safety aspects to validate its usage in human clinical trials and also to develop this as a product that fulfils all domestic and international regulatory guidelines. Hence, in vivo safety data is absolutely essential to validate this product. Therefore, the current study aimed to assess the skin irritation potential of a polyherbal anaesthetic formulation on a single application in Wistar albino rats. The rats were dermally exposed to the polyherbal anaesthetic formulation and after that any change in each application site was recorded at 1 h, 24 h, 48 h, 72 h, 7th day and 14th day. The dermal reactions, which are normally defined as erythema and edema, were assessed based on the scoring system for skin reactions. In the 14-day observation period, our formulation did not induce any responses on intact skin sites after the application. So, the primary skin irritation index of the polyherbal anaesthetic formulation was recorded to be 0.0, which seemed to fall under the category of non-irritant in the experimental rats. Furthermore, no treatment-associated mortality was observed in this study. In addition to this, normal weight gain was recorded in all rats. Hence, it can be concluded that polyherbal anaesthetic formulations are non-irritants and safe on rat skin. Further study on this topic is needed to provide more specific evidence to support the findings.

Keywords: erythema, polyherbal anaesthetic formulation, skin irritation toxicity

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Shan Sasidharan,[1,3] Kaveri Nair A,[2] Sithara MS,[3] Hareendran Nair J[4]

[1]Department of R&D, Pankajakasthuri Herbal Research Foundation, India
[2]Department of Shalyatantra, Pankajakasthuri Ayurveda Medical College & P.G. Centre, India
[3]Small Animal Research Centre, Department of Toxicology and Pharmacology, CARe KERALAM Ltd, India
[4]Pankajakasthuri Herbal India Pvt. Ltd., India

Correspondence: Shan Sasidharan, Pankajakasthuri Herbal Research Foundation, Thiruvananthapuram, Kerala, India, Tel 9188325339 Email drshansasidharan@yahoo.co.in

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Introduction

The Central Council of Indian Medicine's recent notification on 2020 making it mandatory for postgraduate students of two streams of Ayurveda (Shalya Tantra and Shalakya Tantra) to be trained in various types of modern surgical procedures as part of their curricula has sparked a nationwide debate. While biomedical scientists express their displeasure with the decision, Ayurvedic practitioners are seen defending it. As we know, in order to perform surgery, Ayurvedic professionals require an ideal anaesthetic agent along with antimicrobial and wound healing drugs. Unfortunately, there is no ideal anaesthetic agent and antibiotics in Ayurveda for effectively conducting surgery.[1] Moreover, the Ayurvedic professionals depend on modern medicine while conducting the surgeries. To resolve the above-mentioned problem, we, Pankajakasthuri groups, initiated a project to develop herbal-based anaesthetic agents (injection, local and spray) as a supporting aid for Ayurvedic professionals to conduct surgeries. Now we are happy to announce that we are very successful in developing a novel polyherbal anaesthetic agent. The novel polyherbal anaesthetic formulation was formulated using the hydroalcoholic extracts of *Syzygium aromaticum* (clove), *Myristica fragrans* (nutmeg), *Nardostachys jatamansi*, *Acmella calva* etc. After formulating the anaesthetic agent, we have subjected it to the In-Process Quality Control Tests (IPQC) and finished product QC. Subsequently, we subjected the anaesthetic to preclinical and clinical studies to establish its safety and efficacy.

Local anaesthetics (LAs) are used in clinical settings to provide anaesthesia and analgesia after surgery or to treat other acute and chronic pain conditions; they only last a few hours.[2,3] In earlier years, medicinal plants have also been used to produce anesthesia. Cocaine, the first local anesthetic, instigates from a specific plant alkaloid and has been extensively used as an anaesthetic for several years.[4]

The new drug formulation requires a suitable toxicology assessment before being administered to humans and animals.[5] The prediction of numerous side-effects is an important issue in the Registration, Evaluation, Authorization and Restrictions of Chemicals (REACH) inventiveness on chemicals in the preclinical study of medications.[6] As we know, animal skin is highly vulnerable to the majority of the chemicals, thus all novel drug preparations must be checked on animal skin for a definite period of time to know if any skin irritation or erythema is occurring or not.[6,7] Thus, dermal irritation and skin sensitization studies are essential components of a minimum set of toxicity screening that provides a detailed description of the potential hazards of new polyherbal anaesthetic formulations developed by us.[8] But currently, the information based on the studies on dermal irritation and skin sensitization according to the relative toxicology guidelines on polyherbal anaesthetic formulations is still lacking. It is essential to assess the risk of polyherbal anaesthetic formulations.

Thus, the current investigation was intended to examine the safety profile of polyherbal anaesthetic formulations through skin irritation toxicity testing. The testing procedures include mainly observation of mortality and changes in body weight after application. Furthermore, this procedure includes the variations in general symptoms, especially in the skin site after the administration of the formulation in Wistar rats.

Materials and methods

Formulation of polyherbal local anaesthetic formulation

The novel polyherbal anaesthetic formulation was formulated using the hydroalcoholic extracts of *Syzygium aromaticum*, *Myristica fragrans* and *Nardostachys jatamansi*, *Acmella calva*.

Test animals

The female wistar albino rats were utilized in the investigation. Ethical approval to conduct this experiment was gained from the Institutional Animal Ethics Committee (IAEC) with reference no. CKL/TOX/IAEC/2021-2/159.

Laboratory conditions and housing of experimental animals

This study was performed in a Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved institute by strictly following all ethical principles mentioned in the guidelines for animal care. The Institutional Animals Ethics Committee (IAEC) of CARe KERALAM, Trissur, Kerala, India has given its approval for this study (CKL/TOX/IAEC/2021-2/159). About 200 g of Wistar albino rats were used for this investigation. The animals were fed *ad libitum* throughout the acclimatization and study period. Pelletized lab rodent feed (manufactured by the Feed Plant of Kerala Veterinary & Animal Sciences University's School of Animal Nutrition and Feed Technology) was provided.

Acute dermal toxicity test of novel polyherbal local anaesthetic formulation

Preparation of rats for dermal toxicity testing

Three wistar rats were used for this investigation. Each rat's patch was allocated to one of two locations on the dorsal portion of the trunk. One patch was used to apply the positive control and the other was used to apply the test polyherbal anaesthetic formulation. Around 24 hours before the study began, all hair was carefully removed from the dorsal part of the trunk of all test animals by cutting without abrading the skin. Based on their appearance, only animals with a healthy, undamaged epidermis were chosen for the experiment.

Study design

The experiment was strictly done by following the OECD guideline 404.[9] Approximately 24 h prior to the test, hair on the dorsal area of the trunk of the rats were removed by close clipping. Animals were treated in the following manner.

Group	Study particulars	Dose
Group 1	Initial test	1 animal 0.5 ml Polyherbal anesthetic formulation topically
Group 2	Confirmatory test	2 animals 0.5 ml Polyherbal anesthetic formulation topically

In the initial test, up to three test patches were used sequentially to the rats. The initial patch was withdrawn after three minutes. After one hour, a second patch was applied at a different location and removed. Because the observations at this stage suggested that exposure could be safely extended to four hours, a third patch was applied and removed after four hours, and the response was graded. The outcome observed in the above experiment was again established by using two additional rats, each with one patch, for an exposure period of four hours. The residual test chemical was removed at the end of the exposure period, which is normally 4 hours, without affecting the existing response or the integrity of the epidermis.

To confirm the reversibility of the effects of the study drug, the rats were observed for 14 days after the removal of the patches. All rats were observed for the signs of erythema and edema. The responses were scored at 60 minutes, and then at 24, 48 and 72 hours after the removal of the patch. For the initial test in one animal, the test site was also examined immediately after the patch had been removed. Furthermore, the degree of erythema, eschar, and edema formation was observed and evaluated at 24 and 72 hours after administration of the polyherbal anaesthetic formulation, and the primary irritation index (P.I.I.) was calculated. The toxicity of skin irritation was determined using Table 1, 2.[10,11]

$$P.I.I\ (Primary\ irritation\ index) = \frac{Sum\ of\ mean\ (24\ and\ 72h\ readings)}{(Number\ of\ test\ sites = 12) \times (Scoring\ \text{intervals} = 2)}$$

Table 1 Scoring system for skin reaction

Reaction	Irritation score
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
Edema formation	
No edema	0
Very slight edema (barely 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
Maximal possible score for irritation	8
*Other adverse changes in the skin sites shall be recorded and reported	

Table 2 Primary or cumulative skin irritation index categories

Mean Score	Response category
0.0 to 0.4	Negligible/Non-irritant
0.5 to 1.9	Slight
2.0 to 4.9	Moderate
5.0 to 8.0	Severe

Administration of test item

About 24 h prior to the experiment, fur was clipped on both sides of the spinal column (approximately 10 cm × 15 cm). The test chemical to be tested was applied in a single dose to the skin of an experimental animal; untreated skin areas of the test animal served as the control. The test chemical was applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch, which was held in place with non-irritating tape. The test chemical was first applied to the gauze patch, which was 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 test chemical on the skin.

Body weight, food and water intake

The body weight of each rat was recorded prior to treatment on Day 1 and weekly thereafter up to the terminal day of the investigation. The amount of food and water consumed by rabbits in each cage is measured and recorded weekly beginning on the first day of treatment.

Results

Clinical signs and mortality

No clinical signs of toxicity and mortalities were observed in the rats after the administration of the test sample. In addition to this, the

test sample had no adverse effect on the various behavioral responses of the rats up to 14 days of observation period.

Body weight, food and water intake

In the current investigation no treatment related changes in body weight were recorded in test rats (Table 3). No significant differences were noted in food and water consumption throughout the experiment.

Table 3 Body weight and percentage weight gain in rats

Animal no.	Sex	Body weight (g)			Body weight gain (%)	
		Day 1	Day 7	Day 14	1-7 days	1-14 days
1	Female	205	210	210	2.44	2.44
2	Female	195	195	205	0	5.13
3	Female	195	200	205	2.56	5.13

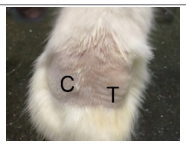
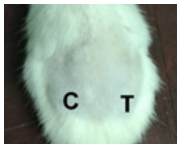
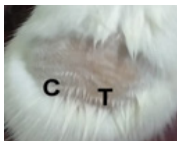
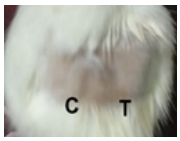
Skin reactions

The dermal irritation scores are presented in Table 4. In rats treated with a single application of the Polyherbal anaesthetic formulation during the study period, no visible skin irritation or inflammation (edema and erythema) was recorded when compared to controls. The acute single exposure dermal irritation combined irritation index score was found to be '0'.

Discussion

Risk assessment is based on risk and exposure data of various chemicals, especially those through animal studies. This procedure is the major stepwise testing method for novel substances introduced into the market with regard to the scientifically comprehensive data about the irritation of the substance.[12] It is well established that herbal products are safe for administration for longer periods of time, but the dearth of toxicological studies on these herbal products raises numerous authentic concerns about the potential toxic effects of chronic use.[13] Thus, quality control for efficacy and safety of herbal products is absolutely required to address the concerns raised by society, especially regarding chronic usage. This investigation was carried out to assess whether the novel polyherbal anaesthetic formulation could cause dermal irritation and skin sensitization in the experimental rats.

Table 4 Dermal reaction scores of Polyherbal anaesthetic formulation

Time after treatment and Lesion Scores	Rat Numbers						Mean score		Figures
	1		2		3				
	Female		Female		Female				
	T	C	T	C	T	C	T	C	
1 h									
Erythema Score	0	0	0	0	0	0	0	0	
Edema Score	0	0	0	0	0	0			
24 h									
Erythema Score	0	0	0	0	0	0	0	0	
Edema Score	0	0	0	0	0	0			
48 h									
Erythema Score	0	0	0	0	0	0	0	0	
Edema Score	0	0	0	0	0	0			
72 h									
Erythema Score	0	0	0	0	0	0	0	0	
Edema Score	0	0	0	0	0	0			
7 Days									
Erythema Score	0	0	0	0	0	0	0	0	
Edema Score	0	0	0	0	0	0			
14 Days									
Erythema Score	0	0	0	0	0	0	0	0	
Edema Score	0	0	0	0	0	0			

Allergy is usually categorized as edema and erythema and is usually triggered by hypersensitivity of the skin or an extreme immune response to an antigen by the body.[14] Edema is an accumulation of extra fluid among tissue cells, while erythema is redness of the skin or mucous membranes caused by hyperemia of superficial capillaries.[15]

In the current study, a single application of a polyherbal anaesthetic formulation to wistar rats caused no dermal irritation or reactions such as erythema or edema . The primary or cumulative skin irritation index of Polyherbal anaesthetic formulation was recorded to be 0.0, indicating that it was non-irritant. From the initial day of the experiment until the end, none of the experimental rats displayed any clinical signs or obvious signs of toxicity in the dermal irritation experiment (single and repeated administration). For up to 14 days, no treatment-related unusual behavior was observed in the Polyherbal anaesthetic formulation-treated rats.

During the 14-day study period, there was no rat mortality. The loss of body weight is a vital marker of gross toxicity of the test material in the experimental animals. This is because drastic toxicity will interfere with nutrient absorption, which will be directly reflected as body weight loss.[16,17] From the initial day of administration of the test material to the end of the experiment, there were no statistically noteworthy mean weight differences in body weights recorded in the control and treated animal groups. Banerjee et al. (2013) reported that body weight loss will be reflected in severe cases of toxicity due to its interference with nutrient absorption.[16] As said earlier, in the present investigation, there was no significant difference in rat body weights or weight gain was recorded in the experimental animals. Hence, it can be expected that the novel Polyherbal anaesthetic formulation has no tendency to induce tissue destruction or interfere with nutrient absorption in the experimental animals. This was supported by the food and water consumption in the treated and control groups, which were nearly identical, with no significant differences.

Conclusion

Outcomes from this investigation clearly demonstrated that Polyherbal anaesthetic formulation was non-irritant to the skin of rats on single application. The overall observations indicated that Polyherbal anaesthetic formulation did not cause any severe inflammatory changes in given skin irritation test. There were no significant changes in body weight, food and water intake were recorded in the rats following a 14-day observational period. Based on the results, it can be concluded that the Polyherbal anaesthetic formulation is safe for topical administration. Further investigation in the areas of subacute toxicity effects of Polyherbal anaesthetic formulation are soon to be published, to confirm the safety before using in clinical therapy.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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