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EVALUATION OF ACUTE DERMAL IRRITATION AND CORROSION EFFECTS OF *BOSWELLIA SERRATA* (BOSWEGEX®) FOR TOPICAL APPLICATION IN HEALTHY NEW ZEALAND WHITE RABBITS

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ABSTRACT
Boswellia serrata (Boswegex®) is a herbal extract that has been standardised for treating pain and inflammation. The objective of this research was to assess Boswegex®'s skin irritation and corrosion in New Zealand white rabbits. The effect of each treatment of Boswegex® was recorded for 14 days on the rabbits' dermally exposed skins. During the observation period, clinical signs and body weight were also examined in addition to the evaluation of the skin reactions (erythema and edema) in accordance with local toxic effects. The reactions were evaluated after 60 minutes, then at 24, 48, and 72 hours after the patch was removed, and the animals were further examined for erythema and edema. In a single exposure test, Boswegex® did not cause any reactions on healthy skin spaces after its application and the subsequent 14-day observation period. Thus, Boswegex® could potentially be regarded as non-irritating and secure for use on rabbit skin. Therefore, topical Boswegex® could be applied to the skin without causing any sensitivity.

KEYWORDS: *Boswellia serrata* (Boswegex®), New Zealand white rabbits, Skin corrosion, Skin irritation.

INTRODUCTION
The largest human organ, the skin, primarily functions as a protective covering for the body. The skin is made up of several layers and is essential for survival in a number of ways. The role of the skin involves defending the body against environmental dangers like caustic or toxic chemicals. The skin can be exposed to chemicals, which can have a number of harmful health impacts. Direct skin effects, immune-mediated skin effects, and systemic effects are the three main categories of chemical-skin interactions that can take place.[1] The terms "skin irritation" and "corrosion" describe localized harmful effects brought on by topical skin contact with a chemical. A chemical or pharmaceutical compound's skin irritability must be assessed in order to ensure its safe use. There are *in vivo* and *in vitro* experiments available to establish the possibility of irritation caused by these substances' contact with human skin. The public is informed about each of the potential risks during exposure through studies on the skin-itching effects of chemicals on experimental animals. The OECD test guideline 404 describes the most commonly used test, the rabbit skin irritation test, which was first described by Draize et al. in 1944.[2] In the skin irritation test, the raw materials or test product were applied to the rabbits' shaved skin, and after 14 days, the skin was examined for any indications of skin irritation. The appearance of a rash, swelling, scaling, inflammation, and abnormal tissue growth in the affected area are the primary symptoms of skin irritation.

The topical side effects of natural goods include phytophotodermatitis, allergic contact dermatitis, and irritation contact dermatitis. Therefore, it is crucial to research the *in vivo* skin toxicity of herbal products. Toxicological research is used to determine whether or not a novel drug should be used in medicine.[3] Evaluation of irritancy potential of any formulations/chemicals to human skin is an essential requirement in the cosmetic sector and pharmaceuticals for topical application.[4]

The *Boswellia serrata* (Salai/Salai guggul) is a medium-sized to big branching tree that grows in dry mountainous areas of India, Northern Africa, and the Middle East.[5] It belongs to the family Burseraceae. There are 600 species in the family Burseraceae, which is widely distributed over all tropical climates, and 17 genera.[6] The pentacyclic triterpenic acids, also known as boswellic acids (BAs), are the primary phytochemicals responsible for the resin's biological effects, including its anti-inflammatory properties. The α-BA, β-BA, 3-O-acetyl-α-BA, 3-O-acetyl-β-BA, 11-keto-β-boswellic acid (KBA), and 3-O-acetyl-11-keto-β-

boswellic acid (AKBA) are the six main boswellic acids (BAs) found in *B serrata* resin. The anti-inflammatory activities of the Boswellia gum resin extracts are believed to be most effectively modulated by the BAs, KBA and AKBA. The anti-inflammatory effects of the Boswellia gum resin extracts are believed to be most effectively modulated by the BAs, KBA and AKBA. They prevent the primary pro-inflammatory enzyme 5-lipoxygenase from producing leukotriene, which is thought to be the fundamental cause of the anti-inflammatory effects of Boswellia extracts).[7,8] Moreover, the production of pro-inflammatory cytokines is also decreased by AKBA and KBA, which is controlled by nuclear factor kappa B.[9–11]

According to research, not all herbal remedies are secure. For instance, research has shown that the usage of several traditional plants might cause adverse effects like skin necrosis.

The purpose of this study is to use *in vivo* experiments to examine the acute cutaneous irritation or corrosion caused by *Boswellia serrata* (Boswegex®). Our Boswegex® is standardised and contains 65% of boswellic acids. Therefore, Boswegex® is intended to treat a variety of dermatological disorders.

MATERIALS AND METHODS

Preparation of Boswegex® with Regular boswellic acids

An aqueous ethanolic extract of the gum resin of *Boswellia serrata* was used for manufacturing Boswegex®, which contains Regular boswellic acids. Boswegex® with Regular boswellic acids were standardised to contain 65% by HPLC, in order to maintain quality and batch-to-batch consistency.

Dried gum was ground into a coarse powder and then extracted using aqueous ethanol. The extraction process was repeated two to three times. The mixed extracts were then filtered under vacuum, and the filtrate was then heated in a rotary evaporator to between 50-60^{o}C to create a viscous extract. The resinous portion of the extract was then removed by washing, and after that, the organic acids were selectively precipitated by treating with alkaline water and then acidification. A semi-dried cake was then produced by decolorizing, filtering, and washing in water with a neutral pH. To make powder, this was further dried at 50–60°C in an oven with a vacuum. By using HPLC, this product was further standardised to 65% of boswellic acids.

Characterization of boswellic acids (Boswegex®) by HPLC

Extraction and purification processes were standardized for Boswegex®. The analytical method was standardized based on HPLC. Briefly, HPLC analysis was performed using a Phenomenex Luna C18, 250 × 4.6 mm, 5 µl column with a flow rate of 1 ml/min in a Shimadzu liquid chromatography equipped with a UV/Vis Detector at a wavelength of 210nm and 254nm to detect the peaks. The sample was eluted by injecting 20 µl of mobile phase (Acetonitrile: Water: Acetic acid (90:10:0.1)) with a run time of 35 min. The peaks 1 to 6 represented 11-keto-β-boswellic acid (KBA), 3-O-acetyl-11-keto-β-boswellic acid, α-boswellic acid, β- boswellic acid, 3-O-acetyl- α-boswellic acid, and 3-O-acetyl-β-boswellic acid, respectively (Fig. 1).

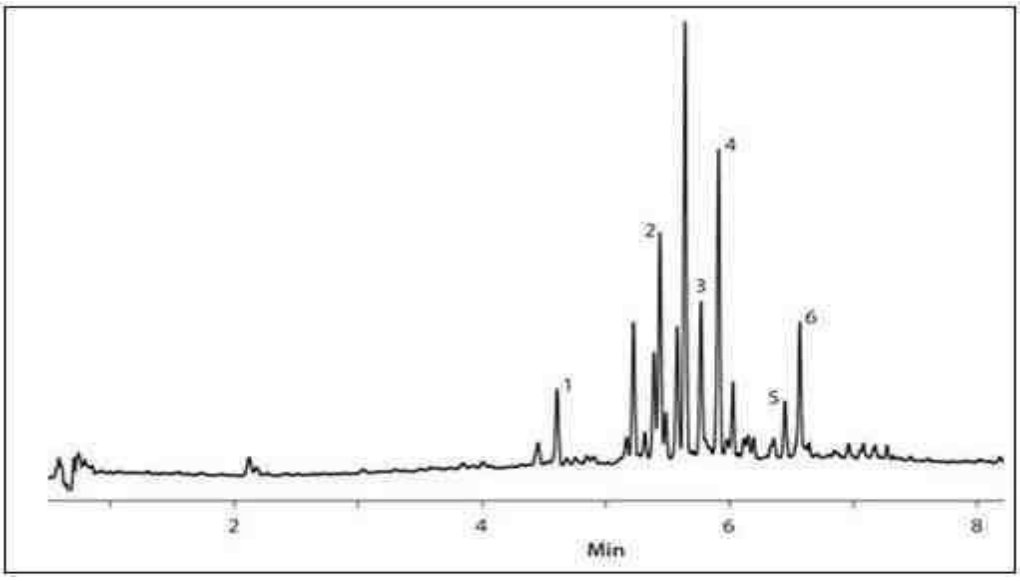

Fig. 1: A representative high-performance liquid chromatography image shows the elution profile of the major boswellic acids in Boswegex® at 210 nm. The peaks 1 to 6 represent 11-keto-β-boswellic acid, 3-O- acetyl-11-keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, 3-O-acetyl-α-boswellic acid, and 3-O- acetyl-β-boswellic acid, respectively.

Chemicals and reagents

Demineralized water (171132) was bought from Karnataka Fine Chem, India. HI Media in India delivered picric acid (0000175497).

Experimental animals

Healthy, female white New Zealand rabbits were obtained from the animal house of Radiant Research Services Pvt. Ltd., Karnataka, India. Animals were housed in groups under standard laboratory settings with a temperature of 22±3°C, a relative humidity range of 30–70%, and a cycle of light and dark lasting 12 hours. Before the experiment began, the animals were acclimated to the laboratory environment to help them get used to the new setting and recover from the stress they had experienced throughout the journey. The animals received unlimited food during the acclimation and research periods. Radiant Research Services Pvt. Ltd.'s Institutional Animal Ethics Committee (IAEC) gave its approval for this specific study to be conducted. The animal experiment was carried out in accordance with CPCSEA Registration Number 1803/PO/RcBi/S/2015/CPCSEA, which was the committee's instructions for the regulation and supervision of animal experimentation. The study director and veterinarian were promptly alerted in the event that adverse reactions suggested pain or distress. According to the veterinarian's expert assessment and, when suitable, the study director's feedback, the animals were either treated or placed to death. In accordance with the situation and in consideration of the CPCSEA Pain, Distress, and Protocol Guidance, treatment or euthanasia was selected.

Experimental design

For this investigation, healthy young adult female rabbits (10–12 weeks old) with body weights between 1000 and 1250 g were chosen as the subject animals and prepared. An electric clipper was used to remove all fur from the dorsal region of the animals' trunks 24 hours before applying Boswegex®. This was done carefully to prevent skin damage that would change its permeability.

Experimental methods

Boswegex® was applied to a small piece of skin (6 cm^2), covered with a gauze patch, and secured with non-irritating tape. Every rabbit received a topically applied dose of 0.5g. The remaining Boswegex® was washed away with water after an exposure period of four hours.

Initial Test

Initially, only one animal was used in the test. The animal received up to three test patches that were all applied in succession. After three minutes, the first patch was removed. A second patch was placed at a different location and taken off after an hour. Four hours after the first two patches were removed, a third one was put on, and the response was assessed. After any of the three consecutive exposures, there was no corrosive effect observed. After the last patch was taken off and the animal was watched for 14 days, no corrosive effect was noticed.

Confirmatory Test

Since there was no evidence of a corrosive impact in the original test, two additional animals were employed to confirm an irritating or negative response. Each animal received one patch application of Boswegex® for a four-hour exposure time.

Observation Period

After the patches were taken out, animals were watched for 14 days to determine whether any effects were still present. During the observation period, it was also possible to observe local toxic effects, clinical symptoms, and body weight.

Clinical Observation

The reactions were assessed at 60 minutes, then at 24, 48, and 72 hours after patch removal, and all animals were inspected for indications of erythema and edema. The test site was also checked right away following the removal of the patch for the initial test on one animal. The following pattern of grading was used to classify and record dermal reactions:(Table 1).

Table 1: Clinical Observation Pattern to Classify Dermal Reaction.

Erythema and Eschar Formation	Score	Edema Formation	Score
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well defined erythema	2	Slight edema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 mm)	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4	Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

RESULTS AND DISCUSSION

Assessment of the dermal irritation

Assessment of the dermal irritation or corrosion of Boswegex® is a significant step in the evaluation of its dermal toxicity. Boswegex® at a dose of 0.5 mg in the initial test did not show any sign of toxicity at the site of application to the skin. Similar observations were recorded even in two additional animals from the confirmatory test. The weights of individual animals in the tested groups are shown in Table 2. The results showed a normal increase in body weight from day 0 to day 14. The Score for grading skin reactions was calculated for each rabbit. Scores for erythema and edema at 60 minutes, 24, 48, and 72 hours were summed,

as were the number of observations for the treated sites. Edema and erythema with a score of 0 were found in all the animals on sites with Boswegex® (Table 3). Acute dermal toxicity test results indicate that none of the test animals exhibited any overt clinical signs of toxicities.

Table 2: Rabbit Body Weight During the Study Period.

Body weight (gm)						
Sample: Boswegex®						
Animal ID	Test	Dose (gm)	Before	After Application		
			Day 0	Day 3	Day 7	Day 14
RB 01	Initial	0.5	1100	1170	1260	1410
RB 02	Final	0.5	1160	1250	1350	1490
RB 03	Final	0.5	1140	1210	1350	1450

Table 3: Individual Score on the Skin Following Application of Boswegex® to the Rabbits.

Grading of Skin Reactions								
Sample: Boswegex®								
Animal No.	60Min		24 Hrs		48Hrs		72 Hrs	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
RB 01	0	0	0	0	0	0	0	0
RB 02	0	0	0	0	0	0	0	0
RB 03	0	0	0	0	0	0	0	0
Score	00	00	00	00	00	00	00	00

0=No reaction

Herbs are considered a significant source of potentially beneficial substances for the development of novel medicinal drugs because they are often safe with no adverse reactions. Topical administration of creams or gels at pathological locations offers considerable advantages in drug release directly to the site of action when compared to ointments.[12,13] Although it is generally accepted that herbal remedies are safe to use for longer periods of time, the absence of toxicological research on medicinal plants raises real worries about the potential side effects of prolonged use.[14] Quality control tests must be carried out for these preparations in order to ensure the efficacy and safety of herbal products.

Edema and erythema are the symptoms of allergy, which is characterized by skin hypersensitivity or an overly strong immunological reaction to an antigen.[15] Erythema is the redness of the skin or mucous membranes induced by hyperaemia of superficial capillaries, whereas edema is the accumulation of significant excess fluid between tissue cells.[16] In the current investigation, a single application of Boswegex® to rabbits did not cause any skin irritation or responses like erythema or edema.

Boswegex® was evaluated for its effect to irritate the skin, and the results showed that there was no dermatological reaction and no redness, deems, or irritation upon application. Over the 14-day trial period, there was no rabbit mortality. The loss of body mass is a crucial indicator of toxicity. There was no discernible variation in the body weight of the rabbits in the current investigation. Therefore, it is safe to say that Boswegex® has no propensity to destroy tissue and seems to have no impact on nutritional absorption.

CONCLUSION

The results of the study showed that Boswegex® did not irritate the skin of rabbits. After a 14-day observation period, there were no discernible changes in body weight. Boswegex® is safe for continuous topical use, according to these findings.

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