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Evaluation of Acute Dermal Irritation, Sensitization and Acute Dermal Toxicity of Leather Cream in Laboratory Animals

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ABSTRACT: Unused leather products attract fungal invasion and deteriorate the product lifespan. The development of antifungal leather cream/polish can solve this array considered that the prepared formulation is skin friendly. Possible adhesion of leather cream to the skin may provide a route for entry of hazardous and harmful ingredients. In this study, leather cream prepared in the laboratory and a marketed product of leather cream were tested for acute dermal irritation in rabbits, skin sensitization in guinea pigs, and acute dermal toxicity in rats. Draize dermal irritation scoring system was used to examine the condition of erythema and edema in rabbits at intervals of 24, 48 and 72 hours. Percentage sensitization rate, sensitization grade, sensitization classifications were accessed in guinea pigs after 24 hours of dermal exposure. Rats were observed daily for 14 days to detect any signs of irritation, change in general behavior, toxicity, morbidity and mortality. Histological examination of rat's skin was performed at the end of the study. No any kind of dermal responses or any observable signs of systemic toxicity were detected in rabbits treated with either of the leather cream samples. No sensitization, erythema or edema was observed in guinea pigs. In the case of rats, the skin was normal with negative signs of toxicity, erythema or edema. Histological study also failed to show any damage in skin tissue layers of rats applied with test materials. The result provides a base for development of anti-fungal leather cream/polish which can enhance the life of leather and avoid the fungal invasion upon storing for a period of time.

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INTRODUCTION

Natural substances like wax and tallow have a history of hundreds of years to be used as leather polish. Only in the 20th-century modern polish formulas were first introduced and many of those formulations can still be found in use. At present, leather polishes are usually made by mixing different natural and synthetic materials, such as naphtha, turpentine, gum arabic and dyes [1-2]. Leather cream is a product with the primary purpose of polishing, shining, waterproofing, and restoring the appearance of leather products like leather shoes, belts, bags, etc. Leather cream overall protects from various possible degradation, increases the life span and helps for the attractive appearance of leather products [1].

Cream is a semi-solid emulsion that contains dispersion of two immiscible/ partially miscible liquids with each other, in which one of the liquids is uniformly distributed as fine droplets (known as dispersed phase) throughout the other liquid (known as continuous phase). Conventionally, the two immiscible phases are often considered as 'oil phase' and 'water phase'. Out of two phases, one is non-polar (oil/lipid/wax) and the other one is polar (water/aqueous solution) [3]. Emulsifier maintains the emulsion's state of dispersion over the extended time after agitation has been stopped. The emulsifying agent/ emulsifier imparts both kinetic and thermodynamic stability [4].

Appropriate toxicology evaluation of new products and substances should be conducted before it is used in human and animal, especially substances which are used daily [5]. Skin is

a vulnerable target organ system as it has a significant role in the entry of hazardous chemical and physical agents. Generally, repeat-dose dermal toxicity studies are carried out for new products or substances using different laboratory animals to characterize the irritation potential and cutaneous/systemic toxicity associated with topical administration of such products, and thus obtained results are used for prediction of human response [6].

Allergic contact dermatitis (ACD) is a type of hypersensitivity reaction that is caused by exposure of exogenous chemical/physical agents in skin. Acute, subacute, and chronic forms of ACD are prevalent. The acute form can be characterized by the presence of erythema and edema which is generally followed by tiny vesicles formation and crusted, weeping lesions. In case of chronic contact dermatitis, lichenified, fissured, or pigmented skin is observed followed by episodes of oozing and crusting [7-9].

Footwear dermatitis is mostly caused by leather processing chemicals, metal buckles, black dyes of shoes and socks, adhesives, plastic, rubber shoes and polishing agents. The risk of occupational skin diseases at tanneries were investigated by many authors and found out that they were mainly related to the chemical exposure of the worker's skin in hot and humid condition [10]. Human skin has a high possibility of exposure during the application, use, and disposal of such products. Adhesion of the product to the superficial organs has the potential to be a risk for many toxicological effects. In this study, the sample product and marketed product were tested for acute dermal irritation in rabbits, skin sensitization in guinea pigs, and acute dermal toxicity in white Albino rats as per the Organization for Economic Cooperation and Development (OECD) and *Committee for the Purpose of Control and Supervision of Experiments on Animals* (CPCSEA) guidelines.

MATERIALS AND METHODS

Preparation of Leather Cream

Standard ratios of bees white wax, cannauba wax and ozokerite was used as oil phase and distilled water as an aqueous phase for leather cream preparation. Proper heating and mixing were carried out at a temperature above the melting point of waxes. Other ingredients incorporated were silicone oil, linseed oil, Sudan black dye, nigrosine black dye and Triton X-100 [1,11]. All the materials used were of analytical grades. The cream was checked for specifications provided by the Bureau of Indian Standards [12].

Acute Dermal Irritation, Sensitization and Acute Toxicity Studies

Acute Dermal Irritation Study

The acute dermal irritation/corrosion study was carried out according to the OECD Guideline 404. Twenty-four healthy rabbits were used which were checked for intactness of skin beforehand. Only those with intact skins were used for the study. The rabbits were divided into four groups:

(i) Positive control group (n =6) received standard irritant (0.8% w/v aqueous solution of formaldehyde),
(ii) Control group (n =6) received distilled water,
(iii) Marketed Product group (n= 6) marketed leather cream
(iv) Test product group (n=6) received the laboratory based sample of leather cream.

On day 0, hair was trimmed and about 25 cm^2 area was shaved on the back dermal surface of each rabbit. Each rabbit was kept on separate cage undisturbed for three days. On day 1, rabbits were treated with standard irritant, distilled water, marketed product or sample product which were evenly applied on the shaved area of rabbits of the respective groups. After 72 hours of exposure, the test materials were removed from the test site and rinsed with distilled water. Draize dermal irritation scoring system (**Table 1**) was used to examine the condition of erythema and edema in the test animals at intervals of 24, 48 and 72 hours [13-14].

Table 1: Draize dermal irritation Score [14]

Draize dermal irritation Score	Level of erythema or edema
0	No erythema or edema
1	Barely perceptible erythema or edema
2	Well-defined erythema or slight edema
3	Moderate to severe erythema or moderate edema
4	Severe erythema or edema

Cage side observations were carried out daily for any signs of clinical toxicity throughout the study. Also, Primary irritation index (PII) was calculated as:

PII= (Sum erythema 24/48/72 hours + Sum edema 24/48/72 hours) / 3×Number of animals

Classification of irritants was done according to the Draize method of classification using the PII scoring (**Table 2**).

Table 2: Classification of Primary irritation index [14]

Primary irritation index	Class of irritant
<0.5	Non-irritant
<2	Slightly irritant
2-5	Moderately irritant
>5	Severely irritant

Skin Sensitization Test

The skin sensitization test was carried out according to OECD Guideline 406 and was modified as per the Buehler method. Twenty-four healthy guinea pigs were used for the study. On day 0, the guinea pigs were divided into 4 groups: a positive control

group where n=6 and received 0.1% w/v 1-chloro-2,4-dinitrobenzene (CDNB) in 10% propylene glycol as a standard skin sensitizing agent, a control group (n =6), a test product group (n=6), and a marketed product group (n=6). The left flank of each guinea pig was shaved. Only those animals with no injury or irritation of the skin were used for the test. On day 0, 0.1% w/v of a sensitizing agent, CDNB, was evenly spread over the shaved skin. Reactions were assessed at 24 hours after application. The intensity of all skin reactions was graded on a sensitization score of 0 to 3, with 0 indicating no reaction, 1 indicating scattered mild redness, 2 indicating moderate and diffuse redness, and 3 representing an intense skin reaction that included erythema and edema with eventual deeper skin damage. Percentage sensitization rate, sensitization grade, sensitization classifications, and sensitization reaction (**Table 3**) were also evaluated [15-17].

Table 3: Rate, Grade, and Classification of Sensitization [15]

% Sensitization Rate	Sensitization Grade	Sensitization Classification
0-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
76-80	IV	Strong
81-100	V	Extreme

Acute dermal toxicity study

Acute dermal toxicity study was conducted in Wistar albino rats according to OECD Guideline 402. Healthy Wistar albino rats of both sexes (12 males and 12 females) weighing between 160 to 280 g were divided into four groups; Group A (control group), Group B (Wax base was applied), Group C (Sample Product), and Group D (Marketed product was applied). Each group consists of 3 male and 3 female animals. On day 0, hair from the back of rats was trimmed with scissors and shaved. Rats were kept on separate cages and left undisturbed for 24 hours. On day 1, respective materials were applied to shaved surface evenly. Rats were returned to their cages. Observation was done two times daily for 14 days to detect if any signs of irritation, change in general behavior, or possible mortality was detected.

Throughout this acute dermal toxicity study, all the animals were observed daily for any signs of irritation, general behavior change, toxicity, morbidity, and mortality.

At the start of the study and once a week (7th and 14th day), body weights were measured. Food consumption (g/kg body weight/day) of each individually caged rat was measured at the start of treatment and weekly up to the 14th day after the application of leather cream [18, 19].

On day 15, rats were sacrificed, and histopathological examination of skin was performed at the end of the study. The skin tissue which was exposed to test material in this study was dissected immediately after the sacrifice of the rats and washed thoroughly with saline water and stored in 10% formalin. All the collected tissues were washed in running water for half an hour and dehydrated in ascending order of alcohol (70%, 80%, 90%, absolute) for two hours in each concentration. The tissues were then cleared in xylene after complete dehydration and embedded in paraffin wax. Finally, serial sections of 5 µm thickness were cut and stained with hematoxylin and eosin for histopathological investigation [20, 21].

RESULTS AND DISCUSSION

Acute dermal irritation study in rabbits

The results of acute dermal irritation test in rabbits are presented in **Table 4**. No kind of dermal responses, including erythema or edema was detected in rabbits treated with either of the leather cream (sample as well as marketed) compared to the negative control group. The PII was calculated as 0 in all of the control, sample and marketed product groups. But in the group applied with 0.8% weight/volume aqueous solution of formaldehyde, all rabbits showed severe erythema 72 hours after the application. The PII was calculated to be 9.99 in this group, indicating severe irritation. Daily observation could not detect any kind of observable signs of systemic toxicity in the sample and marketed group.

Skin sensitization test in guinea pigs

Sensitization score, rate, grade, and classification of test materials and positive control group after 24 hours of dermal application in guinea pigs are shown in **Table 5**. Positive dermal sensitization was achieved using the positive control group (0.1% w/v CDNB). No sensitization was observed in guinea pigs applied with test material and control group. No erythema and edema were observed.

Acute dermal toxicity study in rats

Wistar albino rats were divided into 4 groups: Group A (control), Group B (wax base), Group C (sample product) and Group D (marketed product group). A total of 3 rats/sex/group were used.

Clinical observation and mortality

The skin of all the study animals was normal, and no signs of toxicity, erythema or edema were detected. Also, the locomotor behavior was observed normal throughout the study period.

Terminal bodyweight trends

No significant change in terminal body weights were found in animal groups applied with test materials compared to control group animals. No significant differences in mean body weights were observed between the test and control groups throughout the experiment (**Figure 1**). This result indicates that the exposure to leather cream did not affect the change in body weight animals.

Food consumption trends

No significant difference in food consumption was found among the test and control groups animals throughout the experiment (**Figure 2**).

Table 4: Acute dermal irritation study of leather cream

Positive Control (0.8% w/v Aqueous Solution of Formaldehyde) (n =6)						
Skin Reactions	Erythema			Edema		
	Observation time (h)					
	24	48	72	24	48	72
Total Score	23	20	18	12	9	8
Mean Score	7.66	6.66	6	4	3	2.66
Total of mean score	29.98					
PII	9.99					
Remarks	Severely irritating					
Negative control (n = 6)						
Skin Reactions	Erythema			Edema		
	Observation time (h)					
	24	48	72	24	48	72
Total Score	0	0	0	0	0	0
Mean Score	0	0	0	0	0	0
Total of mean score	0					
PII	0					
Remarks	No irritation					
Formulation (n =6)						
Skin Reactions	Erythema			Edema		
	Observation time (h)					
	24	48	72	24	48	72
Total Score	0	0	0	0	0	0
Mean Score	0	0	0	0	0	0
Total of mean score	0					
PII	0					
Remarks	Non irritating					
Marketed Product (n =6)						
Skin Reactions	Erythema			Edema		
	Observation time (h)					
	24	48	72	24	48	72
Total Score	0	0	0	0	0	0
Mean Score	0	0	0	0	0	0
Total of mean score	0					
PII	0					
Remarks	Non irritating					

Table 5: Sensitization score, rate, grade, and classification after 24 hours of dermal application in guinea pigs

Test material	Sensitization rate (%)	Sensitization grade	Sensitization classification	Reaction
Positive control (0.1%w/v CDNB in 10% propylene glycol), n=6	100 (6/6)	V	Extreme	Intense
Negative control, n=6	0 (0/6)	I	Weak	No
Formulation, n=6	0 (0/6)	I	Weak	No
Marketed product, n=6	0 (0/6)	I	Weak	No

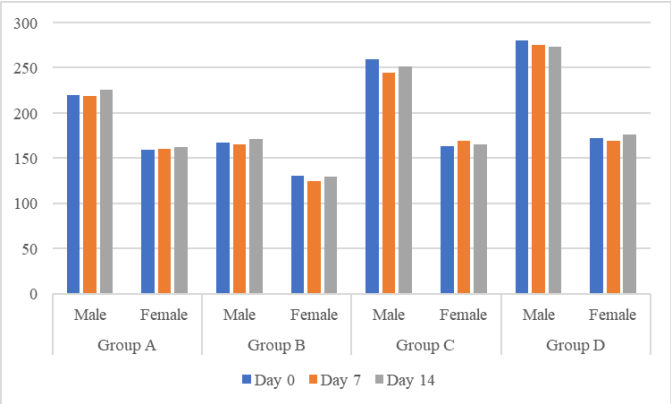

Figure 1: Group Mean Terminal Body Weight (g) of Rats for 14 days

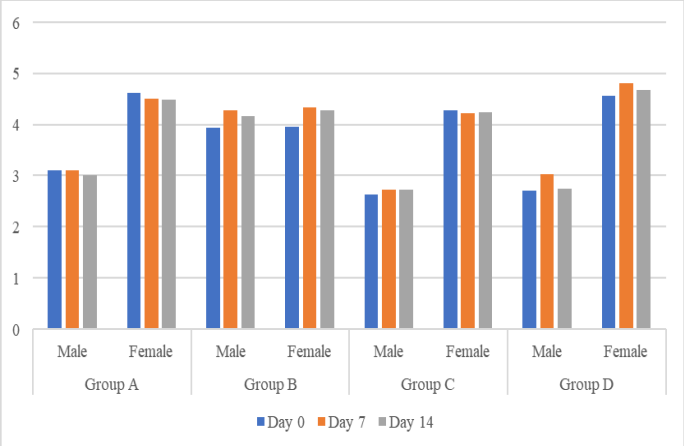

Figure 2: Group Mean Food Consumption (g/kg body weight/day) Trends for Male and Female Wistar Rats for 14 days.

Histology of skin tissue

Histological study showed no damage in skin tissue layers of Group B, Group C, and Group D rats when compared with control group Group A rats (**Figure 3**). From this study, we can be assured that no dermal toxicity was caused by the leather cream samples.

CONCLUSION

Neither of leather creams (leather cream prepared in the laboratory and marketed product) showed any skin irritation, skin sensitization or dermal toxic effects following dermal exposure. No significant changes were detected in body weight and food consumption patterns of both male and female Wistar rats during 14 days of observation period. The result can be extrapolated in the development of concomitant polish/cream which can be used incorporating anti-fungal agent for prolonging lifespan of leather avoiding the fungal damage upon storing.

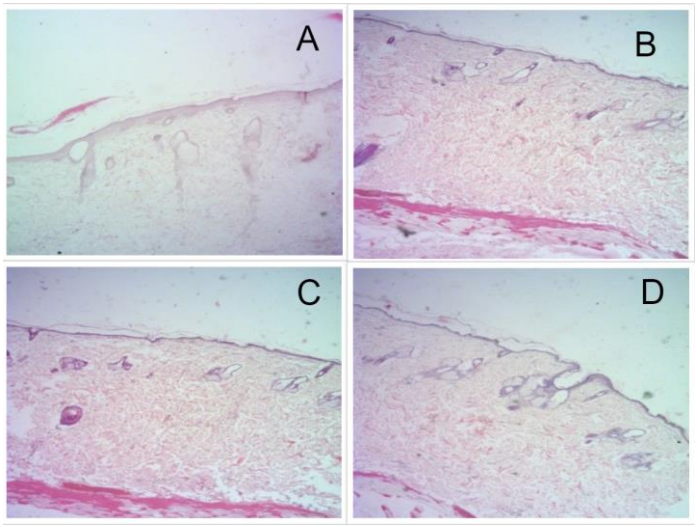

Figure 3: Histological morphology of rat's skin tissue by HE staining of (A) control group rats (B) Wax base group, (C) Test material group (D) Marketed product group.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY

Not declared.

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