

# Perform Skin Irritation Study Of The Developed Formulation

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## Abstract

The goal of this study was to assess the likelihood of Acute Dermal Irritation due to Mastilep gel using OECD criteria. Substances and Techniques: To combat mastitis in ruminants, a new herbal ointment called Mastilep gel has been created for topical use to the udder. It is the suggested method for collecting scientifically solid data on the corrosivity/irritancy of a novel chemical via a series of sequential tests. There were three female rabbits utilized in the experiment. Every specimen was its own control. The degree of irritation or corrosion after using Mastilep gel was measured and recorded. The use of Mastilep gel did not result in any noticeable skin lesions or erythema. Mastilep gel was shown to have no irritating properties, according to the findings. In summary, MASTILEP gel may be used without worry. As Butea monosperma extract cream and gel showed just a little irritating characteristic, they may be safely utilized as topical preparations to treat different skin conditions or as topical cosmetics.

**Keywords:** Mastilep gel, OECD, Acute Dermal Irritation, Skin irritancy, Rabbits, Butea monosperma Gel and Cream

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## INTRODUCTION

Increased somatic cell count in milk and pathological alterations to the mammary tissue are hallmarks of mammary gland inflammation, often known as mastitis. To treat mastitis in cattle, a new method called Mastilep gel may be given to the teats. Most chemicals have a strong effect on animal skin, thus any novel formulations must be tested on animals for a certain amount of time to see whether they cause any irritation or erythema. Some of the skin reactions that might occur while using natural products include allergic contact dermatitis, irritating contact dermatitis, and phytophotodermatitis. Thus, in vivo studies of Mastilep gel's skin toxicity are crucial.

Several variables, such as exposure concentration, duration, frequency, exposed skin location, rate of penetration, and inherent hazardous potential, contribute to skin irritation as a frequent adverse consequence in humans. All chemicals or formulations used in the cosmetics business must undergo testing to determine their potential to cause skin irritation. This is necessary in order to assess the potential for skin irritation from coming into touch with these substances. The shaved skin of rabbits is used in this experiment, and the skin responses are graded based on physiological observations of the animals. The compounds being tested may be either raw ingredients or final formed goods.

The Butea monosperma, also known as the "Flame of the Forest" or the "Palas tree," is used to cure gout, leprosy, and other skin illnesses, and to alleviate burning sensations in the Ayurveda medical system. Glycosides, linoleic acid, flavanoids, etc. [2] were found in high concentrations during chemical analysis of the plants. It's clear the plant offers therapeutic promise for a variety of diseases in which free radicals have been implicated as a causative component. Anti-inflammatory, antibacterial, antifungal, and wound-healing properties have all been shown in earlier investigations of the plant.

While many people make use of *Butea monosperma* for medicinal purposes, no one has yet investigated the plant's cosmetic potential or developed effective formulations. There has been a huge uptick in the popularity of cosmetics made entirely of natural materials. Gels and creams containing *Butea monosperma* extracts were developed for their anti-aging, skin-lightening, and wound-healing properties with this objective in mind. The current research aimed to assess the preliminary skin irritation testing on rabbit and on healthy human volunteers to ensure its usage in cosmetics and the treatment of different skin conditions is safe.

## LITERATURE REVIEW

Parasuraman S, Balamurugan S, Vanishya R. (2018), Drug administration via the skin is both an attractive and difficult study area since the skin is one of our biggest organs. Several age groups employ the dermal formulations for aesthetic and medicinal objectives. Hence, The safety data for these preparations is vital, and long-term toxicity testing is required to reduce delayed effects on users/consumers. The worldwide prevalence of moderate hypersensitivity to fatal poisoning associated with cutaneous preparations has grown dramatically in recent years. Hence, in most toxicological frameworks, testing for toxicity is a must for every formulation, including cutaneous preparations. Several dermal preparations are also accessible without a prescription. Preclinical models, ideally using cell lines or animals, may provide crucial information on the safety of these preparations. The goal of this study is to provide a concise summary of the several ways that cutaneous preparations may be tested for toxicity.

A. I. Arshad, S. M. Khan, N. A. Khateeb, A. M. Mahmood, R. M. Sarfraz, and A. I. Sarfraz (2016), A 2% methanolic extract of *Ananas comosus* L. was included in a topical cream, and its effects on irritation potential, melasma, and sebum contents in healthy persons were compared to those of a placebo. Eleven healthy adults between the ages of 20 and 30 volunteered to participate in the study after providing their written informed permission. During 12 weeks, volunteers applied the research formulation to one side of their faces twice daily and a placebo to the other (three months). Several facial skin parameters were measured at baseline and at a two-week interval using photometric instruments in a draft-free environment with controlled temperature and humidity. The patch test results indicated no significant skin irritancy. In addition, statistical analysis shows that compared to placebo, therapy with formulation is more effective in reducing skin irritancy, melasma, and sebum discharges, with maximum reductions of -20.76 0.89, -54.2 0.37, and - 40.71 0.75 percent, respectively, at the conclusion of the trial. When compared to a typical antioxidant, the extract was 92% as effective. Overall, the volunteers had no adverse reactions to the active cream containing fruit extract, indicating that it may be used to treat contact dermatitis, greasy skin, acne, seborrheic dermatitis, and other skin diseases, as well as to desaturate the skin of people, making them more visually appealing. Consequently, additional clinical investigations of these formulations in patients with compromised skin functions, such as contact dermatitis, melasma, and acne vulgaris, will be required to uncover the actual potential of this fruit.

Solomon Tesfaye, et al. (2019), In the traditional and folk medicines of the Ankober District, North Central Ethiopia, *Lavandula angustifolia* is used to cure a wide variety of ailments in both humans and animals. The goals of this study were to determine whether or not *L. angustifolia* essential oil is hazardous to mice when ingested and whether or not it produces skin irritation in rabbits. Using gas chromatography-mass spectrometry, scientists determined that the most abundant components of *L. angustifolia* essential oil were eucalyptol, camphor, gamma-terpinene, and endoborneol. The LD50 value for *L. angustifolia* essential oil was determined to be more than 2000 mg/kg after an acute toxicity test was performed. In the subacute toxicity study, 2000 mg/kg was given orally to each mouse once a day for 21 days. No changes in body weight, metabolic markers, gross abnormalities, water intake, or food consumption were found to be statistically significant ( $p > 0.05$ ). Histopathology examination of the kidneys and livers revealed no gross alterations. Shaved rabbit skin was used in an ointment formulation at 10% strength to test for skin irritation. *L. angustifolia* oil ointment had no effect

on the skin of mice. The results of this toxicity test showed that essential oil from *L. angustifolia* poses no health risks.

M. M. Gatne, K. Tambe, Adarsh and K. Ravikanth (2015), The purpose of this research was to evaluate the potential for Acute Dermal Irritation caused by Mastilep gel in accordance with OECD standards. Substances and Techniques: To combat mastitis in ruminants, a new herbal ointment called Mastilep gel has been created for topical use to the udder. It is the suggested method for collecting scientifically solid data on the corrosivity/irritancy of a novel chemical via a series of sequential tests. There were three female rabbits utilized in the experiment. Every specimen was its own control. The degree of irritation or corrosion after using Mastilep gel was measured and recorded. The use of Mastilep gel did not result in any noticeable skin lesions or erythema. Mastilep gel was shown to have no irritating properties, according to the findings. In summary, MASTILEP gel may be used without worry.

D.M. Sakarkar, S.V. Tembhurne, B.H. More, and S.N. Sakharwade (2013), The Primary Irritation Index (PII) test developed by Draize was used to determine the level of safety. In the Draize test, PII values between 0.07 and 0.13 were recorded, indicating that the gels and creams posed no significant risk to rabbits. After 72 hours, no responses were seen. Human volunteers who were subjected to the maximum concentration of gel (1.5%) and cream (also 1.5%) showed no skin sensitivity in the study. As *Butea monosperma* extract cream and gel showed just a little irritating characteristic, they may be safely utilized as topical preparations to treat different skin conditions or as topical cosmetics.

## **METHODS**

IAEC gave its approval to using animals in this research (MVC/IAEC/30/2014). Three female New Zealand white rabbits were employed, each weighing 2.5 kg and aged 12 months. The acclimation period for the animals was eight days prior to the application. The rabbits were weighed one day before the commencement of the experiment. A unique identifying mark was placed on each animal. A card was placed in each cage with information on the research number, animal number, test formulation, and gender. The housing was standard for the area. The temperature outside was 25 degrees Celsius, and the humidity was around 70%. The animals were kept on a 12 hour light/dark cycle with free access to food and drink.

### **Preparation of the animals:**

Around 24 hours before the experiment, the animals' fur was clipped carefully along the dorsal portion of their trunks, on each side of the spinal cord. No animals with damaged or diseased skin were utilized, and all necessary precautions were taken to protect it. A single dosage of the test drug (MASTILEP, 0.5 gm) was given to a small area of skin on an experimental animal, and the area was then covered with a gauze patch.

The test subject's untreated skin (on the left) is used as a comparison. The test medication was given to rabbits for one hour. In order to offer a thorough assessment, we read and evaluated the irritation/corrosion level (Table 1) 5 at predetermined intervals and elaborated on its nature.

In addition to recording discomfort, we meticulously documented all other systemic and local toxic consequences, including skin defatting and changes in clinical symptoms of poisoning and body weight. Each rabbit's skin patch was documented and thoroughly monitored twice daily. Information was collected at 24, 48, and 72 hours after the patch was removed.

### **Application of gels and creams:**

Shaved areas of skin of around 6 cm<sup>2</sup> were treated with 1 and 1.5% *Butea monosperma* leaf and flower extract

creams and gel as the test ingredients. The gel and cream treatment areas on each rabbit were covered with gauze, and their backs were secured with a non-occlusive bandage. After that, we put the animals back in their cages. Following 24 hours, the test materials and bandage were taken off, and the locations were checked for skin irritation for an additional hour. After the first 24 hours, the locations were observed again at 48 and 72 hours. Skin responses, including erythema and edema, were scored using a predefined algorithm (Table 1).

**TABLE 1: GRADING OF SKIN REACTIONS**

<b>Erythema and Eschar Formation</b>	
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
<b>Maximum possible: 4</b>	
<b>Oedema Formation</b>	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
<b>Maximum possible: 4</b>	

Animals with marked areas served as controls; these animals received 0.5 grams of the cream and gel basis (without extract) and were observed in the same way.

The following formula was used to compute the SPI for each rabbit. The total scores for erythema and edema at 24, 48, and 72 hours were multiplied by the number of observations for the treated areas. Similar methods were used to determine the SPI for the control and test locations.

$$\text{SPI for Each Rabbit} = \sum \frac{\text{Erythema and Edema grade at 24, 48, and 72 hrs.}}{\text{Number of observation}}$$

Both the treatment and control groups consisted of five animals, and the Primary Irritation Index was calculated by subtracting the total SPI values of the two sets of animals. The mean SPI values of the five rabbits were averaged to get the Primary Irritation Index (PII). Based on the PII, the level of irritation was classified as non-existent, mild, moderate, or severe (Table 2).

**Table 2: Response categories of irritation in rabbit**

Category	Primary Irritation Index (PII)
Negligible	0-0.4
Slight irritation	0.5-1.9
Moderate irritation	2-4.9
Severe irritation	5-8

$$PII = \frac{\sum SPI(\text{Test}) - \sum SPI(\text{Base})}{\text{Number of animals}}$$

**Healthy Human Volunteers Studies:** Animal studies showed high compliance with the prepared formulations, and their use in topical cosmetics had already been demonstrated in prior research; In order to ensure the topical gel and cream formulations used in this research are safe, the scientists conducted skin irritation experiments on healthy human volunteers. The patch test was used to evaluate the formulations for their safety. This is how we did the patch test.

**Human patch trials with healthy participants:** Single and repeated patch testing for skin reaction/irritation were conducted on two age groups: 11 healthy young adults (ages 21-25) and 6 mature women (ages 50-65). Before the mixture was applied, the dorsal skin was scrubbed with alcohol at a 70% concentration. The greatest concentration of FEBM gel (1.5%) and cream (1.5%) were applied to patches on the right forearm, whereas the left forearm patch was applied with the corresponding formulations' bases. The treatment was rubbed into the forearm areas that were indicated (5 cm x 4 cm). The surgical dressing was applied to the affected areas and then covered. After 48 hours (single patch test), we removed the patches and cleaned the forearms with physiological saline. Three separate applications of the formula were made over distinct eras (repeated patch test). After 15 minutes, 1 hour, and 24 hours, cutaneous responses such erythema, edema, pruritus, urticaria, skin allergy, and irritation were tracked to determine how severe they were.

**RESULTS:**

**Erythema:** Hyperemia of the superficial capillaries causes erythema, which manifests as redness of the skin or mucous membranes. One third of the rabbits showed redness after 24 hours compared to the control group. No changes were seen after 48 hours. (Table 3)

**Oedema:** Swelling due to excess fluid in the body's tissues is called edema. Even though herbs are Class 7 Novel Anti-Inflammatory Agents, the risk for irritation must be assessed before using them in a novel dermal formulation. No edematous lesions were seen at any point throughout the study (Table 4). No abnormality was detected in the research data collected on the last day of the test compound.

**TABLE 3: Experimental rabbits were evaluated for erythema and eschar formation at several time points.**

Animal ID	Grading and time intervals		
	24 hr.	48hr	72hr
I	1	0	0
II	0	0	0
III	0	0	0

**TABLE 4: EMMA FORMATION IN EXPERIMENTAL RABBITS AT VARIOUS TIMES**

Animal ID	Grading and time intervals		
	24 hr.	48hr	72hr
I	0	0	0
II	0	0	0
III	0	0	0

**Skin observations:**

One of every three rabbits displayed redness at the 24-hour mark, as seen in Fig. 4 when compared to the control rabbits in Fig. 3. There were no noticeable changes after 48 hours. The absence of a statistically significant difference between the control and test groups throughout time periods is shown in Figures 1 through 8. At no point throughout the observations were any other skin lesions seen, such as defatting of skin, unfavorable skin responses, local systemic alterations, etc.



**FIG. 1: CONTROL (0HRS)**



**FIG. 2: TEST (0HRS)**



**FIG. 3: CONTROL (24HRS)**



**FIG. 4: TEST (24HRS)**



**FIG. 5: CONTROL (48HRS)**



**FIG. 6: TEST (48HRS)**



**FIG. 7: CONTROL (72HRS)**



**FIG. 8: TEST (72HRS)**

Following 24 hours of treatment with 1.5% cream and cream base, erythema and edema scores were observed to range from 0 to 1 in all rabbits, while scores for gel and gel base were similarly low (Table 5). There were no cases of erythema scoring 2 or higher (well-defined erythema) for locations treated with cream or gel that also contained their respective bases. Following 72 hours, formulations and its base were found to have eliminated or significantly reduced mild erythemas at the treated areas. At 24 hours, there was no significant difference in the occurrence of edema at sites treated with cream, gel, or their respective bases. After being treated with any of the available formulations, no animal shows signs of developing edema. After 72 hours, no animals have shown erythema or edema. The gels and creams were found to have a main irritation score of 0.07 to 0.13, placing them in the category of minimal irritating.

Table 6 displays the results of the patch test. Both formulations' maximum concentration (1.5% w/v) were shown to cause no irritation or sensitization in single or repeated patch testing on healthy participants.

**Table 5: Inflammation and swelling after using Butea monosperma extract cream and gel**

Rabbit no.	Reactions	Score for skin reaction																	
		Cream (LEBM)						Cream base	Gel (FEBM)						Gel base				
		1%			1.5%				1%			1.5%							
24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr		
1.	Erythema	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	Erythema	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	Erythema	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	Erythema	0	0	0	1	1	0	0	0	0	1	0	0	1	1	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.	Erythema	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6: Human participants' cutaneous reactions to a 1.5% gel and cream in a Patch test

Parameters	Single patch test	Repeated patch test		
		15 min.	60 min.	24 Hrs.
Erythema	Nil	Nil	Nil	Nil
Edema	Nil	Nil	Nil	Nil
Pruritus and Urticaria	Nil	Nil	Nil	Nil
Skin allergy	Nil	Nil	Nil	Nil
Irritation	Nil	Nil	Nil	Nil

**DISCUSSION**

Data on hazards and exposures are essential for risk evaluation. It is the suggested method for producing scientifically valid data on the corrosivity/irritancy of a product prior to its introduction into the market. 8, 9. There are several active compounds in plant extracts that reportedly have toxicological potential. 10. The present experiment examined the effects of the polyherbal gel MASTILEP on the acute dermal irritation of rabbits. The term "dermal irritation" refers to the temporary harm caused to the skin by a test chemical. 11. Mastilep gel showed no evidence of skin corrosivity/irritation, which may be due to the fact that it contains herbal ingredients.

Both the cream and the gel containing the leaves and flower extract of Butea monosperma caused mild irritation after 24 hours of treatment in the main skin irritation test conducted on rabbits. After 72 hours of being exposed to the test materials, no animals have developed erythema or edema. The gel and cream were determined to have an irritation score of 0.07 to 0.13, putting them in the safe category of minimal irritants.

The skin of a rabbit and a human being reacts differently to environmental and chemical factors. The scientific reason for species-specific differences in skin irritation is, however, still a mystery with the exception of a small number of published research that compare findings obtained in animals and people, In order to determine whether or not a substance is irritating, results from rabbit skin tests have been utilized as a benchmark. Some



compounds are more dangerous to rabbits than humans, and vice versa, which may explain Draize's weak performance in this area. This further proves that it is not always possible to directly translate findings from animal studies to those in humans. In addition, the Draize test's reliability is inconsistent. While eschar development is visible with strong irritants, there was also worry for the animal's pain and discomfort.

This research also compared the results of a previous study on skin irritation conducted on rabbit skin with those obtained from studies conducted on human skin. There was no evidence of skin irritation in human subjects, as shown by the findings.

## CONCLUSION

In this skin irritation test, MASTILEP gel was not shown to generate any significant inflammatory changes. Results from a study testing the safety of a cream and a gel containing *Butea monosperma* extract on rabbits and human skin show that neither species showed any signs of irritation. The formulation was shown to be non-irritating in a Draize test conducted on rabbits. Hence, the *Butea monosperma* extract-containing cream and gel may have additional applicability as a safe topical preparation to treat different skin conditions or as a safe topical cosmetic.

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