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Safety and Skin Irritation Study of Topical Analgesic Preparations Using Draize Assessment

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ABSTRACT

Introduction: A cream is defined as a semisolid dosage form containing one or more medicinal substances dissolved or dispersed in a suitable base. Cream preparations require an irritation test to detect skin reactions such as erythema and edema that may be caused by potentially irritating ingredients.
Objective: This study aimed to determine the safety and skin irritation effects of the raw materials or final product of an analgesic cream preparation using the draize method.
Methods: Seven male New Zealand rabbits were divided into two groups. Group I was the control group, with two rabbits using a commercially available cream containing camphor and menthol as active ingredients. Group II was the treatment group, with five rabbits using an analgesic cream containing camphor and menthol as active ingredients. Erythema and edema were observed at 24, 48, and 72 hours.
Results: No erythema or edema was observed in either the treatment or control groups. The irritation index calculation showed a value of 0 (zero), which can be categorized as non-irritating. These results indicate that the analgesic cream composition is safe and does not cause skin irritation.
Conclusion: The analgesic cream preparation has a primary irritation index that is included in the category of not causing primary irritation to rabbit skin topically.

Keywords : Analgesic, Anti-irritant, Cream, Draize

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INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Raja et al., 2020). Pain management typically involves the administration of general analgesics and opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), as well as adjuvant analgesics. These agents are commonly delivered systemically or via the neuraxial route; however, their clinical utility may be constrained by notable adverse effects. Pharmacologically, analgesics exert their primary mechanism of action by targeting specific sites within the central and peripheral nervous systems, thereby enabling high concentrations at peripheral effector locations relative to comparatively low serum levels (Flores et al., 2012). Among the available topical dosage forms intended for pain relief, creams represent one widely utilized option.

Creams are defined as semisolid preparations containing one or more active pharmaceutical ingredients that are dissolved or dispersed in an appropriate base (Thushara et al., 2022). Topical analgesic creams are commonly used to alleviate external pain, including musculoskeletal and joint discomfort. These over-the-counter preparations are practical for repeated application throughout the day, and certain formulations may provide prolonged analgesic effects for several hours post-application, thereby supporting improved pain management (Layber et al., 2025).

Given their direct contact with the skin, cream formulations require irritation testing to evaluate potential dermatological reactions such as erythema and edema, which may arise from components with irritant properties. Such testing is essential to ensure the safety of the formulation prior to its application on human skin (Kristiani & Filadelfian, 2024). Therefore, the aim of this study is to assess the safety profile and potential skin irritation effects of the raw materials or final product of an analgesic cream using the Draize testing method.

MATERIALS AND METHODS

Study Design

This study employed an experimental design using seven male rabbits, which were allocated into six groups. Groups I and II served as control groups and were administered balm. Groups III, IV, V, VI, and VII received the analgesic cream. The study was conducted in the Pharmacology and Toxicology Laboratory of Ngudi Waluyo University, Ungaran.

Materials

The materials used in this study included the analgesic cream with the composition listed in Table 1, New Zealand white rabbits as test animals, 96% ethanol, and balm (containing menthol and camphor) as the control.

Table1. Analgesic Cream Formulation

Composition	Concentration (%)
Champora	3
Menthol	3
Emulgade	15
Glycerin	10
Sodium Benzoate	0,1
Aquadest	Ad 100

Preparation of Test Animals

For the irritation assessment of the analgesic cream, seven rabbits were prepared. The test animals used were healthy New Zealand white rabbits weighing 1.5–2 kg and aged 5–6 months. The

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rabbits were acclimatized for one week under standardized conditions and provided with the same type of feed and water throughout the acclimatization period.

Irritation Test Procedure

Seven rabbits were acclimatized for one week in the animal facility and fed regularly. On the dorsal region of each rabbit, a rectangular area measuring 3 × 2 cm was outlined, and the fur within the marked area was carefully trimmed using scissors and a hair clipper. The skin was then cleaned and disinfected using 96% ethanol.

For the control group, 0.5 g of balm containing menthol and camphor was applied topically to the designated area. For the treatment group, 0.5 g of the analgesic cream was applied topically to the prepared area. After application, the treated skin area was covered with sterile gauze and secured with adhesive tape.

The first observation was conducted 24 hours after application by removing the gauze and adhesive tape, then allowing the skin to be exposed for one hour before evaluation. After the assessment, the area was re-covered, and subsequent observations were carried out at 48 and 72 hours. For each skin condition a score of 0 to 4 is given depending on the severity of the resulting skin reaction (Draize, 1959).

Table 2. Edema Degree Score

Skin reactions	Score
No edema	0
Very slight edema (almost invisible)	1
Well-defined peripheral edema	2
Moderate edema (raised margin ±1 mm)	3
Severe edema (raised margin more than 1 mm and extending beyond the affected area)	4

Table 3. Erythema Degree Score

Skin reactions	Score
No erythema	0
Very slight erythema (barely visible)	1
Clearly demarcated erythema	2
Moderate to severe erythema	3
Severe edema (beet-red) to slight crusting	4

Each irritant sample is calculated based on the sum of the edema and erythema indexes, then the irritation index is calculated as follows, primary irritation index :

$$\frac{amount\ of\ erythema\ 24/48/72\ hours\ +\ amount\ of\ edema\ 24/48/72\ hours\ \ (\text{behavior} - \text{controls})}{number\ of\ rabbits}$$

The irritation index obtained is then compared with the degree of irritation score as follows:

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Table 4. Irritation Degree Score

Evaluation	Score
No irritation	0,0
Very slight irritation	0,1-0,4
Slight irritation	0,41-1,9
Moderate irritation	2,0-4,9
Severe irritation	5,0-8,0

RESULTS

Irritation Test Results at 24, 48, and 72 Hours

Table 5. Irritation Test Observation Results

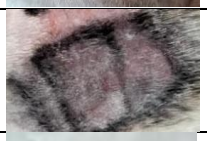
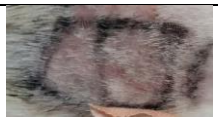
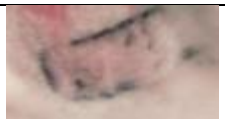
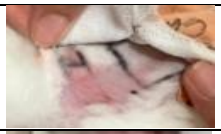
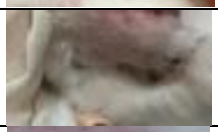
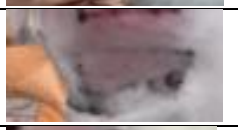
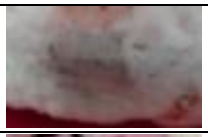
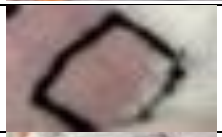
No	Replication	24 hours	48 hours	72 hours
1	Control I			
2	Control II			
3	1			
4	2			
5	3			
6	4			
7	5			

Table 6. Irritation Test Results

Replication	24 hours		48 hours		72 hours		Irritant index
	Erythema	Edema	Erythema	Edema	Erythema	Edema	
Control 1	0	0	0	0	0	0	0
Control 2	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0

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DISCUSSION

In this study, an irritation test was conducted to determine whether or not irritation arose from the analgesic cream applied to the rabbit's skin. Male white rabbits were used because their biological condition is more stable than that of female rabbits, whose biological condition is affected by several factors such as the ovulation cycle, pregnancy, and lactation period. In the irritation test, the parameters observed were the presence of primary irritation effects in the form of erythema (redness), edema (swelling), and skin rashes on the area where the cream was applied (Rasyadi, 2024). The irritation test was conducted on the back skin of male white rabbits, as shown in Table 4.

A cream is a semi-solid dosage form containing one or more medicinal ingredients dissolved or dispersed in a suitable base. The term has traditionally been used for semi-solid preparations with a relatively liquid consistency formulated as a water-in-oil or oil-in-water emulsion (Rusmin, 2021). The analgesic cream used in this study used the active ingredients camphor and menthol as well as additional ingredients emulgade, glycerin, sodium benzoate, and distilled water.

Camphor is a solid, paraffin-like substance that is white and somewhat transparent, characterized by a strong and distinctive aroma. It is a terpenoid compound with the chemical formula $C_{10}H_{16}O$ and contains naphthalene. Camphor is naturally found in the wood of the camphor laurel tree (*Cinnamomum camphora*) (Narendra et al., 2023). Camphor has a counterirritant, rubefacient and mild analgesic action, and is a major component of liniments for relief of fibrositis, neuralgia and similar conditions (Zuccarini & Soldani, 2009).

Menthol is a naturally occurring cyclic terpene alcohol ($C_{10}H_{20}O$). Its use in dermatology is very old and very ubiquitous: cooling, antipruritic, analgesic, antiseptic, etc. Menthol induces a feeling of cold and can thereby reduce the sensation of pruritus. Creams and lotions containing 1–5 % menthol are used since decades for the quick relief of pruritus (Misery, 2016).

Emulsifiers (emulgade) are components added to reduce the coalescence of dispersed droplets in the continuous phase to an insignificant extent. Emulsifiers (surfactants) stabilize by occupying the interface between droplets in the external phase, and by creating a physical boundary around the particles that will coalesce, also reducing the interfacial tension between the phases, thereby improving the emulsification process during mixing. The use of emulsifiers is usually required at 5% – 20% of the weight of the oil phase (Rusmin, 2021).

Glycerin or glycerol is a trihydric alcohol containing the trivalent glycerin radical (C3H5) which is a thick, colorless liquid with a molecular weight of 92, a specific gravity of 1.25 g/cm^3 and has a high boiling point and decomposes at a temperature of 290^{o}C (M. Afif Aufari et al., 2013). Glycerin is a humectant that does not cause irritation, is hygroscopic and can mix with almost all substances. Glycerin functions as a moisturizer and the use of glycerin is more advantageous in terms of appearance because the paste formed will be shinier (Sita et al., 2022).

Sodium benzoate is a salt derived from benzoic acid that is often used as a food preservative (Prayuda et al., 2023). It inhibits the growth of microorganisms such as mold, yeast, and bacteria, and extends the shelf life of products (Hadriyati, 2020). Aquadest or aqua distillata, also known as pure water or distilled water, is a clear, colorless, odorless, and tasteless liquid. Its molecular formula is H_2O with a mass of 18.02 g/mol. In preparations, aquadest is used as a solvent. Store aquadest in a well-closed container.

Observations on the test animals were conducted by applying analgesic and control creams to the skin of the rabbits' backs. Irritation was observed by observing the erythema and swelling (edema) that occurred on the rabbits' skin at 24, 48, and 72 hours. The resulting erythema and edema scores were calculated, and the resulting primary irritation index was calculated. The results of observations of erythema, edema, and the degree of irritation index are shown in Table 5. Observations showed no erythema or edema in either the treatment or control groups. The irritation index calculation showed a value of 0 (zero), which can be categorized as non-irritating. These results indicate that the analgesic cream composition is safe and does not cause skin irritation.

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CONCLUSIONS

Based on research results, analgesic cream preparations made from camphor and menthol were proven not to cause skin irritation in New Zealand white rabbits with an irritation index score of zero. Subchronic or chronic irritation tests are necessary to assess the safety of using anti-irritation creams for repeated and long-term use, as the primary irritation test only describes acute effects.

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Funding Source

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Availability of data and materials

This study was conducted on only one sample in the treatment group containing the active ingredients camphor and menthol. Future research could include irritation testing using analgesic cream preparations containing camphor and menthol at various concentrations to achieve more significant results.

Authors' contributions

There are 2 researchers, where the main researcher (Indah Kurniawati) plays a role in preparing research tools and materials as well as conducting research and writing articles, while the research member (Devi Mardiyanti) plays a role in conducting research and processing research data.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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