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Topical anti-inflammatory and analgesic activities of *Laportea decumana* (Roxb) *Wedd* extract cream in rats

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ABSTRACT: *Laportea decumana* is a plant that has traditionally been used to treat pain. This study aimed to determine the anti-inflammatory and analgesic activity of *L. decumana* leaf extract (LD extract) topical cream in rats. The anti-inflammatory effects of LD cream at a concentration of 0.5%, 1%, and 2% extract were determined by carrageen-induced acute inflammation on rat hind paws. Hydrocortisone (1%) cream was used as a comparison. The volume of oedema and IL-6 level were analysed following one day of application. Another set of experiments was performed to assess the analgesic activity of LD cream by intraplantar injection of 1% formalin, using 30% methyl salicylate cream as a comparison. Pain indicators, including foot stamping and licking, were observed for 60 minutes. The results showed that rats received the 2% LD cream had reduced carrageenan-induced paw oedema of up to 13.1% at the 3rd hour, and 8.1% at the 4th hour, compared to the placebo ($p < 0.05$). Rats treated with the 2% LD cream also had the lowest level of IL-6 in their paw tissue, which was comparable to 1% hydrocortisone cream treatment. Similarly, in the analgesic tests, a significant decrease in the amount of foot stamping and licking was seen following the administration of 2% LD cream ($p < 0.05$). This analgesic effect was similar to that of 30% methyl salicylate cream. In conclusion, cream containing 2% LD extract provided anti-inflammatory and analgesic effects in rats, and might be useful for treating pain and inflammation in humans.

KEYWORDS: *Laportea decumana; a*nti-inflammatory; Carrageenan; analgesic; formalin

1. INTRODUCTION

Inflammation and pain are the major, and most common, symptoms of many diseases. Inflammation can be triggered by various stimuli, such as infection, toxic chemicals, and physical damage resulting in redness, swelling, heat, and pain [1]. When inflammation takes place, a process of tissue destruction occurs, which involves blood products such as plasma proteins, fluids, and leukocytes entering into the disrupted tissue [2]. Inflammatory process is characterized by leukocyte activation and infiltration into injured areas, as well as the release of pro-inflammatory mediators, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) [3].

Inflammation often occurs with pain. Pain is an unpleasant sensation associated with tissue damage [4]. Pain involves stimulation of nerve tissue in the brain to produce a response that we perceive to be painful. The mechanism of pain induction involves various sensors, including nociception, peripheral sensitization, phenotypic changes, central sensitization, and ectopic excitability [5].

Laportea decumana (Roxb.) Wedd is an endemic plant of Maluku Island, Indonesia, that has been empirically used to treat pain. *L. decumana* is a shrub that can grow up to 2 m in height. This plant has soft, brittle, well-branched stems, and has fine hairs on the surface of the leaves. According to Simaremare's study [6], *L. decumana* plants contain compounds belonging to the alkaloids, glycosides, steroids/triterpenoids, and flavonoid groups, but do not contain saponins, polyphenols, or tannins. The leaves are believed to have the capacity to relieve aches, fatigue, and stomach pains, but this has not been scientifically proven [7]. This present study aimed to examine the anti-inflammatory and analgesic activity of ethanolic extract of *Laportea decumana* in rats (*Rattus norvegicus*), applied as topical cream at a concentration of 0.5%, 1%, and 1.5% w/w.

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2. RESULTS

2.1. Effect of LD topical cream on carrageen-induced edema

In the negative control, 1 hour after the injection of carrageenan, the rats started to experience noticeable changes in the gross morphology of their paws, including redness and swelling. Red and swollen paws were also evident in the LD-treated groups, especially those treated with 0.5% and 1% LD cream. However, the swelling of the hind paws was less noticeable in those treated with 2% LD cream. Unlike the other groups, the positive control rats did not experience any edema in their paws.

Table 1 shows the percentage of hind-paw edema after injection with 1% carrageenan. The increase in paw edema peaked at 2 and 3 hours in the negative control, 0.5% LD cream, and 1% LD cream groups, but the volume of edema gradually decreased after 4 hours. The percentage of paw edema in the negative controls intensified up to 75.2 ± 14.0% after 3 hours, and only 2% LD cream treatment led to an efficient inhibition of paw edema formation as compared to the negative control. With the 2% LD cream treatment, the percentage of edema at 3 hours markedly decreased to 13.1 ± 7.7%. Indeed, the paw edema in the 2% LD cream-treated group almost returned to normal after 4 hours of carrageenan injection, similar to that seen in the positive control rats treated with 30% methyl salicylate. Meanwhile, the other groups still experienced paw edema after 4 hours of carrageenan injection, even though the extent of the swollen paw decreased over time. The volume of edema in the 2% LD cream group reduced more rapidly than in the rats treated with 0.5% and 1% LD cream ($p < 0.05$). There was no significant difference between the 2% LD cream and the positive control in the volume of paw edema (see Figure 1).

Table 1. The percentage of edema in rat paws 1 to 4 hours after carrageenan injection

Group treatment	% Edema ± SD			
	1 Hour	2 Hours	3 Hours	4 Hours
Negative Control	35.9 ± 9.8	71.6 ± 11.7	75.2 ± 14.0	55.6 ±11.7
LD Cream 0,5%	48.2 ± 1.7	69.5 ± 13.1	78.0 ±16.4	47.9± 9.0
LD Cream 1%	36.6 ± 28.9	103.9 ± 38.9	106.7± 29.2	60.3 ± 44.0
LD Cream 2%	17.4 ± 5.8	45.0 ± 5.3	13.1 ± 7.7	8.1 ± 7.3
Positive Control	9.8 ± 5.3	9.4 ± 4.0	7.8 ± 6.7	6.7 ± 5.9

Negative control: cream base only; LD: *L. decumana* extract; Positive control: 1% hydrocortisone

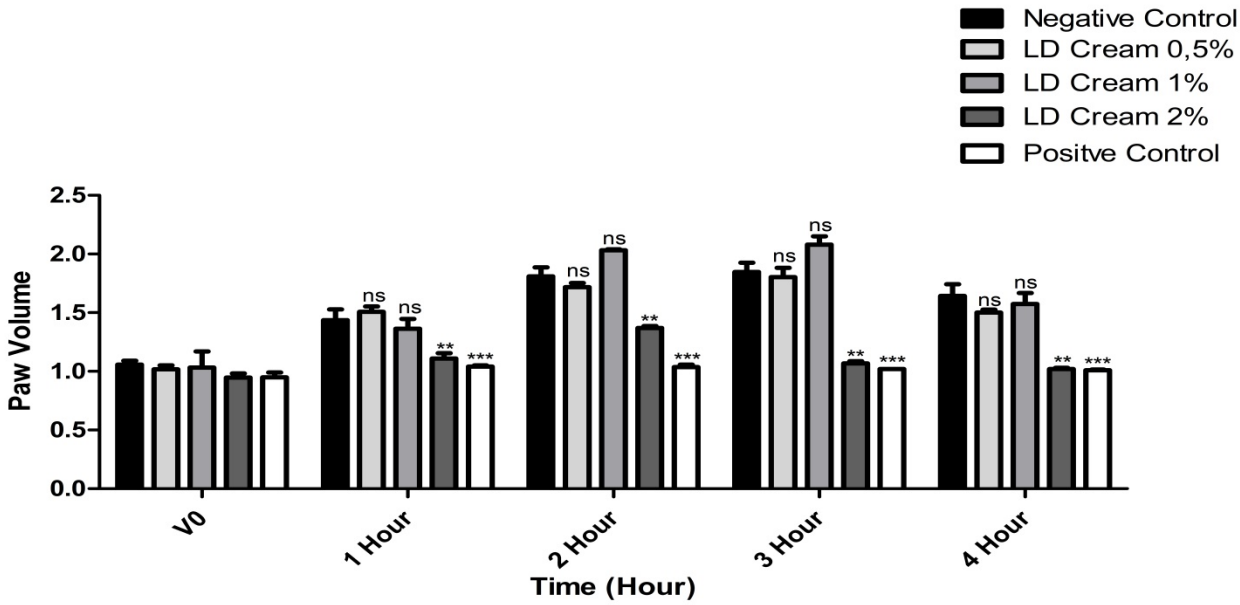

Figure 1. Carrageenan-induced an increase in paw volume of the rats treated with different treatments after 4 hours of injection. (ns = non-significant; ** = $p<0.05$; *** = $p<0.001$)

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2.2. Effect of LD cream on interleukin-6

As shown in Figure 2, carrageenan significantly increased the concentration of IL-6 in the hind paw of rats 4 hours after injection of 1% carrageenan. It is shown that the IL-6 levels in the injected paws were 5.8 ± 1.1 (negative control), 5.6 ± 0.3 (0.5% LD cream), 5.0 ± 0.5 (1% LD cream), 3.3 ± 0.5 (2% LD cream) and 2.4 ± 0.4 p/g paw tissue (positive control), respectively. The levels of IL-6 in paws treated with 0.5% and 1% LD cream did not significantly change compared to the negative controls. However, there was a very significant reduction in IL-6 concentration in the 2% LD cream-treated paws compared to those who were only treated with the cream base ($p < 0.01$). This reduction of IL-6 levels 2% LD cream rats was somewhat comparable to that of the positive control rats, who received methyl salicylate treatment.

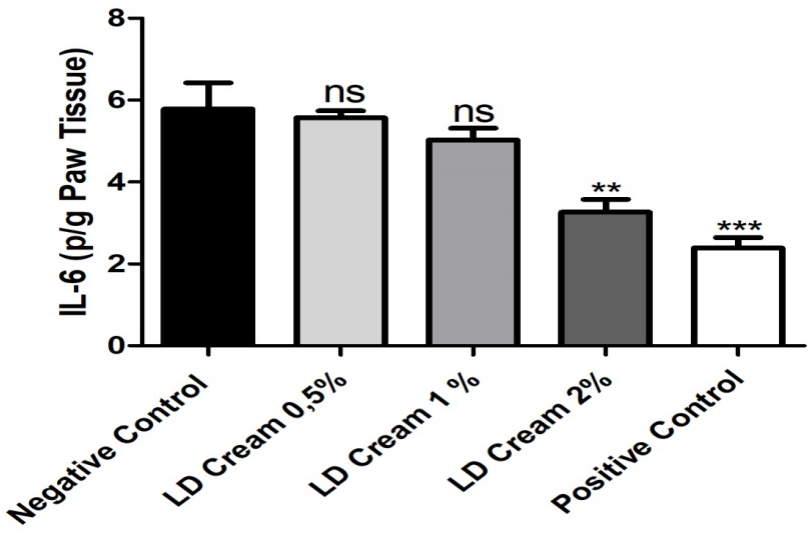

Figure 2. The levels of IL-6 in paw tissue after 4 hours injected with 1% carrageenan (ns = non-significant; ** = p<0.01; *** = p<0.001)

2.3. Effect of LD cream on formalin-induced analgesic

The administration of 0.5%, 1%, or 2% LD cream 30 minutes before the injection of formalin had an analgesic effect that significantly reduced the amount of paw licking in rats. Table 2 shows that the LD cream pretreatment led to a significant inhibition of licking (%protection of licking) by 63%, 73%, and 92% at a dose of 0.5%, 1%, and 2%, respectively. Methyl salicylate as a positive control had a %protection of licking of 92%, comparable to that of the 2% LD cream.

Table 2. Paw-licking response and percentage of protection of paw licking within 10 minutes of formalin injection in rats.

Treatment Groups	Number of licking ± SD	% Protection
Negative Control	12.67 ± 6.11	0
LD Cream 0.5%	4.67 ± 0.57	63
LD Cream 1%	3.33 ± 1.52	73
LD Cream 2%	1.00 ± 1.00	92
Positive Control	1.00 ± 1.00	92

Negative control: cream base only; LD: *L. Decumana* extract; Positive control: 30% methyl salicylate

In Figure 3, it can be seen that within 10 minutes of formalin injection, the mean number of paw-licking responses was 12.67 in the negative control. The treatments with 0.5% and 1% LD cream significantly reduced the paw-licking response in rats post injection of formalin ($p < 0.05$). The paw-licking responses were even

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lower in rats treated with 2% LD cream compared to the negative control ($p < 0.01$), which was similar to those of the positive control.

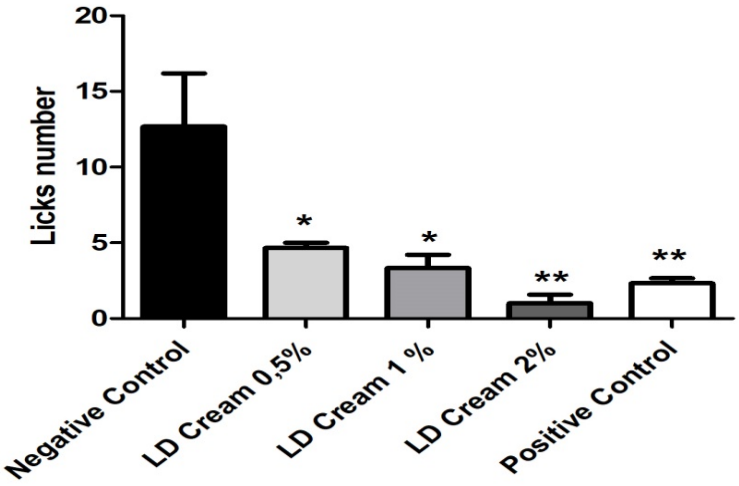

Figure 3. The mean number of paw-licking response in rats within 10 minutes of 1% formalin injection (* = $p<0.05$; ** = $p<0.01$)

It is shown in Table 3 that the number of foot stamps in the negative controls was 341.7 ± 37.87. With the 0.5% and 1% LD cream pretreatments, the rats still produced regular stamping, and this was not statistically different from the negative controls. In contrast, pretreatment with 2% LD cream caused a significant reduction in foot stamping, which was almost half (53% protection) of that which occurred in the negative control rats ($p < 0.05$). In the positive control, the percentage of stamping protection was 69%, but it was not statistically different from the group receiving 2% LD cream treatment.

Table 3. Paw-stamping response and percentage of protection of paw stamping within 60 minutes of formalin injection in rats

Treatment Groups	Number of stamping ± SD	% Protection
Negative Control	341.7 ± 37.87	0
LD Cream 0.5%	356.0 ± 38.20	4
LD Cream 1%	249.3 ± 97.51	27
LD Cream 2%	159.7 ± 11.72	53
Positive Control	105.3 ± 32.59	69

Negative control: cream base only; LD: *L. Decumana* extract; Positive control: 30% methyl salicylate.

Figure 4 illustrates the number of stamping responses within 60 minutes of formalin injection. A very significant difference was found in the number of stamping responses between the negative control and the 2% LD cream treatment group (0.05), as well as the positive control ($p < 0.01$). Lower concentrations of LD cream (0.5% and 1%) did not significantly affect the amount of stamping in rats, indicating the same intensity of pain was experienced by these groups after induction by 1% formalin.

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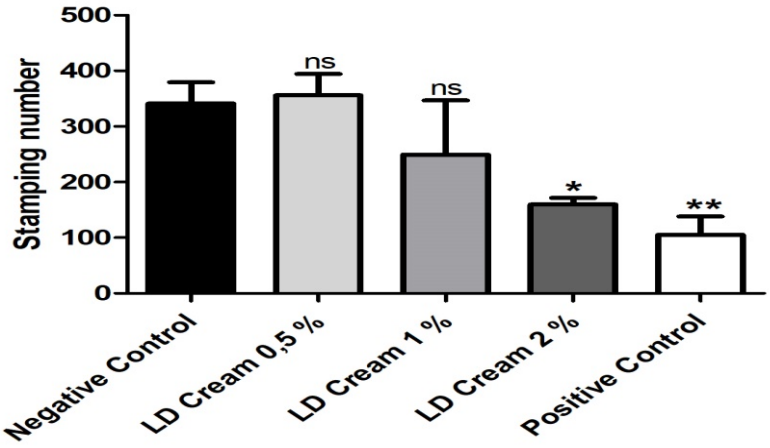

Figure 4. The mean number of paw-stamping response in rats within 60 minutes of 1% formalin injection (* = $p<0.05$; ** = $p<0.01$

3. DISCUSSION

This study showed that the topical application of *L. decumana* cream significantly suppressed carrageenan-induced paw edema, and reduced formalin-induced pain. Intraplantar injection of 1% formalin is an inducer of pain that increases the stimulation of sensory nerves by activating TRPA1 receptors. Formalin can induce a biphasic pain response [8]. In addition, this study showed the presence of mechanisms that suppress pro-inflammatory mediators such as interleukin-6. Inflammation is the main response of tissues damaged due to the induction of various stimuli, such as chemicals, and is characterized by redness, pain, edema, and heat [9]. During the initiation of inflammation, the process of tissue destruction occurs, vascular permeability increases, and leukocytes are activated. Pro-inflammatory mediators are also released, including prostaglandin E2 (PGE2), bradykinin, and cytokines [2], such as TNF-α, which then triggers the release of IL-6, IL-1, and cytokine-induced neutrophil chemoattractant-1 (CINC-1), which stimulates prostaglandin synthesis and releases sympathetic monoamines, respectively [10]. These pro-inflammatory mediators can prompt hyper-nociceptive response, leading to decreased pain threshold and hyperalgesia [10].

Carrageenan injection has been widely used to induce paw edema in rodents [11]. It results in the development of a biphasic swelling pattern. It firstly triggers the first-phase mediators of inflammation, such as leukotrienes, histamine, serotonin, cyclooxygenase, and kinin [12]. Subsequently, in the late phase (after 1 hour), it causes neutrophil infiltration into inflammatory pathways and increases the production of pro-inflammatory mediators, such as PEG2, and various cytokines [13,14]. Carrageenan is also known to increase the production of reactive oxygen species and the release of cytokines, including TNF-α, IL-1β, IL-6, and IL-10 [15,16]. The cytokines will in turn activate the immune system that stimulate leukocytes and provide protection against tissue injury [17].

The results of this study showed that the intraplantar injection of carrageenan into the rats' hind paws produced a severe inflammatory response, resulting in paw swelling. However, pretreatment with LD cream reduced the swelling. Paw swelling was still persistent even after 4 hours in the rats that were treated with the cream base only. In contrast, in the 2% LD cream group, the swell was relieved faster—in as little as 3 hours post injection. However, the anti-inflammatory effect of the 2% LD cream was still not as strong as the 1% hydrocortisone cream (positive control), which successively inhibited the process of swelling from the beginning of its induction. Therefore, swollen paws were not observed from the first hour to the end of the experiment (4 hours) in the positive control.

The results of our study showed that edema caused by intraplantar injection of carrageenan was associated with an increase in IL-6 in rat hind-paw tissue. Topical administration of 2% LD cream showed a significant decrease in IL-6 levels compared to the negative control group ($p < 0.01$). Likewise, the positive control group, which was given 1% hydrocortisone cream, had greatly reduced IL-6 levels in the hindlimb tissue compared to rats that were only treated with the cream base. This indicates that the application of 2% LD cream on paws could inhibit the stimulation of pro-inflammatory mediators.

In this present study, LD cream's analgesic effects were tested against intraplantar injection of formalin in the paws of rats. It is shown that the positive control of methyl salicylate and all LD cream concentration treatment groups (0.5%, 1%, and 2%) showed a significantly lower cumulative number of licking responses

for the first 10 minutes following induction compared to the negative control ($p<0.05$). Furthermore, the cumulative number of foot-stamping responses were also significantly lower in the 2% LD cream treatment ($p<0.05$). Because licking and foot stamping are phenotypic parameters of pain [18], we can infer that the 2% LD cream treatment was able to provide pain protection to the 1% formalin-induced rats that was equivalent to 30% methyl salicylate cream, a standard topical analgesic [19]. The analgesic actions of *L. decumana* are assumed to be due to its secondary metabolites, such as alkaloids, steroids, triterpenoids, and glycosides [20].

Alkaloids elicit analgesic effects by acting on opioid receptors to reduce the perception of pain and emotional responses from the central nervous system (CNS) [20]. Opium alkaloids from plants can resemble pharmacological properties of opioids, hence they also provide analgesic effects through their action on brain regions [21]. To date, the analgesic effect of *L. decumana* has not been adequately explored. However, another species from the genus *Laportea* Gaud. has been previously studied. The analgesic and anti-inflammatory effects of the methanol extract of *Laportea interrupta* have been shown to inhibit the pain of rats whose tails were thermally stimulated. It is believed that *L. interrupta* extract inhibited the pain response due to the presence of opiates (opium alkaloids) in the plants, which reduce the stimulation of pain by acting directly on the CNS [22].

Laportea leaves contain sterols, steroids, and triterpenoids [23]. Unlike alkaloids, steroids act by suppressing the phospholipase enzyme, as a result it inhibits the formation of prostaglandins and leukotrienes [24]. Dongmo et al. indicated that the analgesic effect of *Laportea sp.* may also be derived from the phytosterol contents in the extract, which are associated with the inhibition of prostaglandin synthesis [25].

Indole alkaloids can inhibit the oxidation of arachidonic acid to endoperoxides, reduce lipoxygenase activity [26], and decrease reactive oxygen species formation, leading to a reduction of pain and inflammation [27]. A decrease in lipoxygenase enzyme activity causes the formation of leukotrienes, which in turn will activate leukocytes that stimulate pro-inflammatory pathways [26]. Terpenoids can inhibit arachidonic acid oxidation and subsequently scavenge the free radicals leading to the inhibition of pain response and inflammation [28].

Plants in the *Urticaceae* family have also been found to contain flavonoids and saponins, which are thought to have analgesic effects [29]. The mechanism of action of flavonoids' analgesic activity involves the inhibition of cyclooxygenase enzymes and arachidonic acid generated by prostaglandins, thus reducing pain [28]. Additionally, flavonoids also restrain neutrophil degranulation and inhibit cytokine and free-radical release, as well as the enzymes that contribute to inflammatory reactions [30].

4. CONCLUSION

Based on the results of this study, it can be concluded that 2% *L. decumana* cream can produce anti-inflammatory and analgesic effects in rats. The presence of analgesic and anti-inflammatory activity in LD cream provides scientific evidence for the development of this traditional medicine for pain relief. However, further research is required to determine the specific compounds in *L. decumana* that are responsible for these effects.

5. MATERIALS AND METHODS

5.1. Plant material

L. decumana was taken from the village of Wakal, Maluku Province, in the eastern part of Indonesia. The leaves of *L. decumana* were dried and pulverized before being extracted. The extraction was done by maceration using 70% ethanol for 72 hours with occasional stirring. The filtrate was evaporated using a rotary evaporator. The obtained extract was evaporated with a water bath to obtain a thick extract.

5.2. Drugs, chemicals, and cream preparation

All drugs and chemicals, such as hydrocortisone acetate (First Medipharma) and methyl salicylate (Eagle Indo Pharma), were purchased from a local pharmacy in Makassar. Λ-Carrageenan (Tokyo Chemical Industry) and formalin were obtained from a medical equipment store in Makassar, Indonesia. The cream base was prepared to contain ethyl alcohol (2%), triethanolamine (4%), stearic acid (10%), propylene glycol (10%), liquid paraffin (5%), and hydantoin (0.1%). The thick extract of *L. decumana* was incorporated at concentrations of 0.5%, 1%, and 2% w/w into the cream base. The thick extract of L. *decumana* was incorporated at concentrations of 0.5%, 1%, and 2% w/w into the cream base. The composition of each cream is depicted in Table 4.

Table 4. The composition of *L. decumana* cream

	Composition (%)		
	Formula A	Formula B	Formula C
Extract of *L. decumana*	0.5	1	2
Cetyl Alcohol	2	2	2
Triethanolamine	4	4	4
Stearic acid	10	10	10
Propylene Glycol	10	10	10
Liquid Paraffin	5	5	5
Hydantoin	0.1	0.1	0.1
Aquadest	qs	qs	qs

5.3. Animals

Male albino rats (*Rattus norvegicus*) weighing 150–200 g were used in the study. The animals were adapted for 14 days in a well-ventilated laboratory, and maintained under standard conditions of light, food, water *ad libitum*, and temperature (25°C). The ethical clearance was approved by the Faculty of Public Health, Hasanuddin University, with ethical clearance number 9712/UN4.14.1/TP01.02/2022.

5.4. Carrageenan-induced acute inflammatory model

Five groups of five animals were used in this study. Group I, as the negative control, was given a cream base only; group II was given *L. decumana* ethanolic extract cream at a dose of 0.5%; group III was given *L. decumana* ethanolic extract cream at a dose of 1%; group IV was given *L. decumana* ethanolic extract cream at a dose of 2%; and group V, as the positive control, was given 1% hydrocortisone cream. Induction of edema was achieved by the subplantar injection of 100 µL 1% carrageenan. The cream was gently rubbed onto the plantar surface of the right back foot using the index finger 30 minutes before the carrageenan injection. The volume of paw edema was measured using a plethysmometer at intervals of 1, 2, 3, and 4 hours. The percentage of edema was calculated with the following formula [31]:

$$\%\, Inflammation = \frac{(VT - V0)}{V0} \times 100$$

VT: paw volume at time T (ml^3)
V0: baseline paw volume (ml^3)

At the end of the experiments, the rats were euthanized, and carrageenan-induced paw tissue was dissected. Tissue samples were weighed and homogenized with Phosphate Buffer Saline (PBS) pH 7.4, then centrifuged at 2000–3000 rpm for 20 minutes. The supernatant was stored at -20°C until the IL-6 analysis was performed.

5.5. Formalin test

The formalin test was used to evaluate the analgesic effect of topical *L. decumana* cream. Thirty grams of cream containing 0.5–2% *L. decumana* extract was applied on the back of the right paw using the index finger. Methyl salicylate (30%) cream was used as the positive control, and the cream base was used as the placebo (negative control). After thirty minutes, 50 µL of 1% formalin solution was administered intraplantarly to the rats' right hind paws. The pain response in rats, including foot-licking and foot stamping, was observed directly within 60 minutes by an investigator. In addition, the rats' responses to the formalin injection were also recorded via video to reconfirm the direct observation. %protection is calculated as follows [32]:

$$\%\, protection = \frac{(Na - Nb)}{Na} \times 100$$

Na: average number of stamping or licking in the negative control
Nb: average number of stamping or licking in the treatment group

5.6. Measurement of interleukin-6

Measurement of interleukin-6 levels in the paw tissue was performed using a commercial ELISA kit according to the kit instructions. The absorption was read at a wavelength of 450 nm, and the IL-6 tissue level was expressed as pg/g paw tissue.

5.7. Statistical analysis

The normality of data distribution was tested using Shapiro-Wilk analysis. Comparisons between means of different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The level of significance was defined as $p < 0.05$. Data were expressed as mean ± SD.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

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