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Application of Moringa Oleifera leaves extract cream inhibits paw edema in white male Wistar rat (Rattus norvegicus) induced by carrageenan 1%

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ABSTRACT

Introduction: Inflammation is the body's response to body tissue injury characterized by swelling or edema. *Moringa oleifera* is known to have many benefits as herbal medicine. *Moringa oleifera* leaves have been shown to have anti-inflammatory activity. The polyphenol content in Moringa leaves, such as flavonoids and tannins, has been observed as a potential anti-inflammatory.

Methods: Thirty white male Wistar rats were randomly divided into five groups. Group 0 (negative control group) was given base cream, group 1 (positive control) was given 2.5% hydrocortisone cream, group 2 was given 10% moringa leaves extract cream, group 3 was given 12% moringa leaves extract cream, and group 4 was given 14% moringa leaves extract cream. One hour after treatment was applied in the plantar area, all mice were induced with 0.1 ml of 1% carrageenan. The thickness of the rat paw edema was measured every 1 hour for 6 hours using a caliper.

Results: This study showed a significant difference in rat paw edema between the base cream and the entire treatment groups ($p<0.05$). There was no significant difference between the 2.5% hydrocortisone cream group and the 10%, 12%, and 14% moringa leaves extract cream group ($p>0.05$). The 14% cream extract had the largest paw edema inhibition among other moringa leaves extract creams which were 27.4%.

Conclusion: This study concludes that 10%, 12%, and 14% of moringa leaves extract cream have anti-inflammatory effects with higher concentrations resulting in better efficacy.

Keywords: anti-inflammatory, moringa leaf extract, cream, carrageenan, rat paw edema
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INTRODUCTION

Various skin diseases have an inflammatory basis as their etiopathogenesis. Inflammation is a response to tissue damage due to various stimuli, both chemical and mechanical, also is the body's natural defense to repair damaged tissue. The inflammatory process includes microvascular damage, increased capillary permeability, leukocyte migration to the inflammatory area, and the release of inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, and leukotrienes which clinically appear as erythema, edema, warmth, and pain.[1,2] Edema is characterized by the presence of plasma fluid that moves from the bloodstream to the interstitial tissue at the trauma site.[3] The therapy widely used to treat skin inflammation is topical corticosteroids. Currently, topical corticosteroids are widely abused so that it often causes side effects.[4]

Recently, traditional medicine is being widely used as alternative medicine in developing countries. The World Health Organization (WHO) estimates that 75-80% of the world's population uses herbal medicines for health.[5] Traditional medicine is an ingredient or herb that can come from plants and be used for treatment or health care. Traditional medicine can be extracted in liquid, thick, or dry form.[6]

One of the plants that can be used as traditional medicine is Moringa (*Moringa oleifera)*. Moringa plants are known to treat diabetes, hypertension, hypercholesterolemia, and wound healing. Various scientific studies have proven that moringa leaves contain minerals, vitamins, proteins, lipids, and various other phytochemicals such as sterols, tannins, flavonoids, terpenoids, and quercetin.[5,7]

Moringa leaves have been shown to have anti-inflammatory activities. One of the compounds in moringa leaves that are thought to have anti-inflammatory effects is flavonoids and tannins. The anti-inflammatory mechanism is suspected to be a decrease in oxidative stress, inhibition of the cyclooxygenase enzyme, which leads to inhibition of prostaglandin synthesis, and a decrease in the production of several cytokines.[5,8]

Utilization of the efficacy of moringa leaves developed in various dosage forms

has also begun to be widely studied. The results showed that moringa leaves extract could be applied topically in the form of gels, ointments, and creams. The advantage of giving topical anti-inflammatory drug preparations is that it is easy to use because it can be directly applied to the inflamed area and has fast absorption, therefore, it can directly provide a therapeutic effect. Previous research has conducted an anti-inflammatory test of 5% moringa extract in gel preparations and found that inflammation was reduced by 47.09%.[9] Similar research was also carried out using preparations in the form of cream of Moringa leaves extract with a concentration of 12%, and the results showed that the cream of Moringa leaves extract had a fairly good anti-inflammatory effect.[10] Research conducted by Husni et al. obtained moringa leaves extract cream with a concentration of 10% is a stable and non-irritating cream, but in that study, the anti-inflammatory effect of the 10% moringa leaves extract cream has not been tested.[11] Topical preparations in the form of creams are easier to apply, comfortable to use, non-sticky, and easy to wash with water.

Based on this description, the researchers conducted a study to prove that moringa leaves extract with concentrations of 10%, 12%, and 14% could inhibit edema, which is a sign of acute inflammation in white rats with paw edema technique induced by carrageenan 1%. Carrageenan 1% is a polysaccharide capable of activating inflammatory mediators to induce edema. Based on the study results, it is hoped that information about *Moringa oleifera* leaf extract in cream preparations can provide an anti-inflammatory effect so that it can be used as an alternative to reduce skin inflammation.

METHODS

This research is an experimental study with a post-test-only control group design.[12] This research was conducted in May 2021 at the Histology Laboratory, Faculty of Medicine, Udayana University, and Faculty of Pharmacy, Mahasaraswati University. The samples used were 30 rats (Rattus norvegicus), Wistar strain, male, aged 6-9 weeks, white with a bodyweight of 150-200 grams. The animals used were by the requirements of the experimental research. The samples were divided into five groups, namely group 1 (negative control) given carrageenan 1% and base cream, group 2 (positive control) was given carrageenan 1% and hydrocortisone 2.5% cream, group 3 was given carrageenan 1%, and moringa leaves extract 10% cream, group 4 was given carrageenan 1%, and moringa leaves extract 12% cream, and group 5 was given carrageenan 1%, and moringa leaves extract 14% cream.

Moringa leaves were obtained from Banjarangkan, Klungkung district. Clean moringa leaves are dried by aerating without exposure to sunlight for seven days. A total of 1 kg of dried leaves were ground using a disc mill to obtain them in powder form. Maceration carried extraction by adding 70% ethanol solvent into a container containing moringa leaf powder and then closed for one day (1 x 24 hours) protected from light. After that, while stirring, filtered using Whatman filter paper, then obtained macerate (filtrate) filtrate pulp. The ethanol macerate was combined and evaporated using a vacuum rotary evaporator at a temperature of 45 degrees Celcius until an ethanol extract was obtained from evaporation in the form of a thick liquid, and phytochemical tests were carried out.

The formulation of cream base ingredients: stearic acid 142 grams, glycerin 100 grams, sodium borate 2.5 grams, triethanolamine 10 grams, nipagin 0.1 grams, aquadest 750 milliliters, the ingredients are mixed to form a cream. Moringa leaf extract cream formulations: Moringa leaf extract (10%, 12%, and 14%), stearic acid 142 grams, glycerin 100 grams, sodium biborate 2.5 grams, triethanolamine 10 grams, nipagin 0.1 grams, aquadest 750 milliliters, the ingredients are mixed to form a cream.

A total of 30 rats were adapted for 1 week. Rats were selected randomly and divided into 5 groups, with each group consisting of 6 rats. Before being given treatment, the left hind legs of the rats were marked and measured using a caliper as the initial diameter (Do). One hour before carrageenan induction, the rats' left leg was smeared with 0.2 grams of the test preparation for each group. The negative control group on the left foot was smeared with base cream. The control group was positive, the left foot was smeared with hydrocortisone 2.5%, the treatment group was smeared with moringa leaves extract cream 10%, 12%, and 14%. After 1 hour of administration of the test preparation, the acute anti-inflammatory test treatment was carried out using the Paw Edema technique by injecting 0.1 mL of carrageenan 1% into the left leg of the rats subplantarly. The thickness of the rat paw edema was measured with a caliper every 1 hour for 6 hours. The thickness of the rat leg edema was obtained from the difference in the thickness of the left leg edema of the rats injected with carrageenan 1% and the feet before being induced. From the thickness of the foot edema obtained, calculate the percentage inhibition of inflammation. The rats that have been used for research are returned to the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University.

The thickness of the foot edema was calculated using a digital caliper and documented in the form of photographs. Measurement of anti-inflammatory activity was carried out by measuring the thickness of the edema of the hind paws of mice using a digital caliper. Starting from 1,2,3,4,5 and 6 hours after being induced by 1% Carrageenan. The edema thickness data to be processed is the difference between the foot thickness before and after treatment using the area Under the Curve (AUC) of the thickness of the soles of the feet of rats induced by Carrageenan 1% for each treatment at each measurement period. The presence of anti-inflammatory activity can be seen from the percentage inhibition of inflation and calculated by the following formula:[13]

Inflammation Inhibitory Power (%) =

$$\frac{AUCk - AUCp}{AUCk} x\ 100\%$$

Notes:

AUCk = AUCk mean of AUC thickness of rat paw edema in the negative control group (mm.hour)

AUCp = AUCp average of AUC thickness of paw edema of rats given the test compound with a dose of n (mm.hour)

Descriptive analysis was conducted to determine the characteristics of the data including the mean thickness of rat paw edema. Normality and homogeneity tests were carried out with the Shapiro-Wilk Test and Levene's Test. For the comparison test, normally distributed and homogeneous data were tested using a one-way analysis of variance (ANOVA) with a 95% confidence level to find out if there were differences in the group differences between groups.

RESULTS

Mean Area Under Curve (AUC) and Inflammation Inhibitory Power of Rat's Foot Edema Thickness

The negative control group had the largest AUC of 4.778 mm/hour, which showed the negative control group produced the largest edema among the other treatment groups (Figure 1). The inhibition power of inflammation in each treatment group was calculated using the percentage of inflammation inhibition. The results showed that the positive control group had the greatest inhibitory power of 27.4%, followed by the cream extract group of 14%, which had the inhibitory power of 25.3% (Table 1).

Normality and homogeneity test results

The test results show normal and homogeneous data.

Comparability Analysis of Rat Foot Edema

Comparability analysis was aimed to compare rat paw edema between groups after being given the treatment, which was tested using the one-way (NOVA) test. The mean thickness of rat paw edema in the negative control group was 3.47 ± 0.45 mm/hour, positive control 4.78 ± 0.99 mm/hour, 10% cream extract 4.04 ± 0.28 mm/hour, cream extract 12% 3.75 ± 0.51 mm/hour, and cream extract 14% 3.92 ± 0.73 mm/hour. Comparative analysis of rat paw edema between groups showed that there were significant differences in rat paw edema between 5 groups ($p<0.05$) (Table 2).

To find out the different groups, it was continued with the Post Hoc test using the Least Significant Difference (LSD) test. The results showed a significant difference in edema thickness in the negative control group and the other treatment groups ($p<0.05$). In comparing the positive control group with the other treatment groups, there were no significant differences in the thickness of the rat paw edema ($p>0.05$). The comparison between groups of Moringa leaf extract cream showed a non-significant difference in the thickness of the rat paw edema ($p>0.05$) (Table 3).

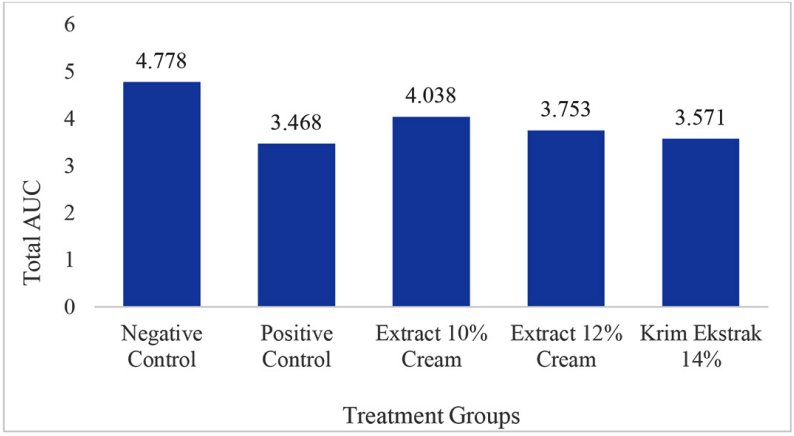

Figure 1. Mean total AUC of each treatment group

Table 1. Inhibition of inflammation in each treatment group

Treatment Group	Power of inflammation inhibitor (%)
Negative Control	0
Positive Control	27,4
Extract 10% Cream	15,5
Extract 12% Cream	21,5
Extract 14% Cream	25,3

Table 2. The difference in mean paw edema of rats between groups after being given treatment

Treatment Groups	Number of samples (n)	Mean ± SD (mm/hour)	P-Value
Negative Control	6	4,78 ± 0,99	0,006*
Positive Control	6	3,47 ± 0,45	
Extract 10% Cream	6	4,04 ± 0,28	
Extract 12% Cream	6	3,75 ± 0,51	
Extract 14% Cream	6	3,92 ± 0,73	

Notes:
*Significant if p<0,05;
Abbreviation: SD, standard deviation

Table 3. Comparison between groups

Treatment Groups	Mean Difference	P-Value
Negative control with positive control	1.31	0.001*
Negative Control with extract 10%	0.74	0.041*
Negative Control with extract 12%	1.03	0.006*
Negative Control with extract 14%	1.21	0.002*
Positive Control with extract 10%	0.57	0.108
Positive Control with extract 12%	0.29	0.413
Positive Control with extract 14%	0.10	0.765
Extract 10% with extract 12%	0.29	0.412
Extract 10% with extract 14%	0.47	0.184
Extract 12% with extract 12%	0.18	0.600

Notes:
*Significant if p<0,05

DISCUSSION

Anti-inflammatory activity testing was carried out to determine the anti-inflammatory power of moringa leaf extract cream. The analysis results after being given treatment showed that the negative control group produced the highest mean AUC of 4.778 mm/hour with 0% inflammation inhibition power. This indicates that the negative control group, namely the base cream, could not inhibit the formation of edema. In contrast, hydrocortisone 2.5% as a positive control had the lowest mean AUC, which was 3,468 mm/hour, which showed that the positive control group had an anti-inflammatory effect with the power of inflammation inhibitory value 27.4%.[14]

Moringa leaf extract cream treatment group 10%, 12%, 14% proved to have anti-inflammatory activity with anti-inflammatory ability, from small to large, respectively: the treatment group moringa leaf extract cream with a concentration of 10% had a total AUC value of 4.038 mm/hour and inhibition of inflammation is 15.5%. Moringa leaf extract cream with a concentration of 12% has a total AUC value of 3.753 mm/hour and inflammation inhibition of 21.5%. Moringa leaf extract cream with a concentration of 14% has the smallest AUC value (3.571 mm/hour) compared to the other two and inflammation inhibition of 25.3%. The smaller the AUC value, the greater the percentage of inflammation inhibition.

Moringa leaf extract cream 10% and 12% were also able to provide an anti-inflammatory effect, although not as good as Moringa leaf extract cream with a concentration of 14%, which provides an anti-inflammatory effect close to positive control. At the percentage value of the inhibition of inflammation by moringa leaf extract cream, it can be seen that along with the increase in concentration, the anti-inflammatory activity is higher so that the ability to inhibit the formation of edema is greater. This follows Clark's theory, the intensity of the effect is directly proportional to the occupied receptor fraction. Therefore, the greater the active dose/drug, the pharmacological effects caused by the test compound will be even greater because the test compound,14 occupy the more receptors

This is supported by previous research, particularly by Ulfa et al., who conducted anti-inflammatory testing using 1%, 3%, and 5% moringa leaf extract gel. The study showed that the administration of the extract with the largest concentration (5%) had the greatest edema reduction effect (47.09%).[9] But, the results of this study are different from research by Nanaryain, who conducted an anti-inflammatory test of Moringa leaf extract cream on rats with the paw edema technique measured using a plethismometer. The study showed that at a concentration of 12%, the anti-inflammatory activity of the cream was 66.84%, but at a concentration of 24%, it had an anti-inflammatory activity of 60.15%.[10] This may be because at a concentration of 12%, it effectively binds to the receptor. The intensity of the effect reaches its maximum when the drug occupies all receptors. Therefore, increasing the dose does not increase the anti-inflammatory effect.[15]

This study showed that the three tested moringa leaf cream extract formulas provided the same anti-inflammatory effect. Based on the results that have been shown, it appears that the increase in rat paw edema at intervals of 1-3 hours and then gradually decreased (data not shown) occurred in the positive control group given hydrocortisone 2.5% due to the presence of glucocorticoids which can inhibit the release of arachidonic acid, so that reduce the production of prostaglandins and leukotrienes.[16] The same response also occurred in the treatment group given moringa leaf extract cream 10%, 12%, and 14% due to the presence of polyphenols in moringa leaves which can suppress inflammation.

In the inflammatory response, oxygen uptake occurs due to oxidative stress, resulting in increased production and release of ROS in damaged areas.[8] Oxidative stress causes inflammatory cells to produce inflammatory mediators that stimulate the formation of COX-2, iNOS, and inflammatory cytokines such as TNF-α, IL-1β, and IL-6.[8,17]

The ability of moringa leaves to suppress inflammation is due to the content of secondary metabolites of polyphenols such as flavonoids and tannins. Nieman's research showed that the aqueous extract of Moringa leaves provides anti-inflammatory activity in vitro with the mechanism of decreasing TNF-α, levels through inhibition of NF-kB.[18] Another study conducted by Sulistyawati found that the ethanol extract of Moringa leaves orally could inhibit COX-2 expression at a dose of 140 mg/kg BW with anti-inflammatory power of 24.30±2.960% was able to reduce COX-2 expression 46.37±6.434% in mice.[19] Research by Arulselvan et al. found that Moringa leaf extract could inhibit the expression of COX-2, iNOS, TNF-α, and IL-1-β in lipopolysaccharide-induced macrophages.[8]

The results of the phytochemical analysis of the content of Moringa leaf extract (data not shown) in this study found the presence of tannin compounds but no flavonoid compounds. Different environmental conditions can cause the difference in the content of these chemical compounds from the place of origin, season, plant genetics, drying method, leaf maturity stage, and the extraction method used.[20]

Tannins are polyphenolic compounds that are abundant in Moringa leaves. The amount of tannin in M. oleifera ranged between 13.2 gTAE/kg and 20.6 gTAE/kg in dry leaves. Tannins have anti-inflammatory activity and antioxidant activity. In the inflammatory process, tannins can inhibit the production of oxidants by neutrophils, monocytes, and macrophages and directly inhibit reactive oxidants such as OH and NO.[21-23] This is supported by the research of Yeo et al., who examined tannins in the form of nanogels that were able to reduce levels of ROS, IL-6, and TNF-α.[24] Another study by Park et al. showed that tannins in the form of epigallocatechin gallate regulate COX-2 expression, which inhibits PGE2 production in macrophages.[25]

Moringa leaf extract cream is expected to act as an alternative skin inflammation treatment. The polyphenolic compounds in Moringa leaves are considered a quite potential anti-inflammatory. This study has been proven that Moringa leaf extract has an anti-inflammatory effect that can significantly reduce the thickness of carrageenan-induced rat paw edema.

This research on Moringa leaf extract cream still has limitations. It has not

investigated the side effects caused by giving the extract topically in the long and short term. However, by obtaining data and information about the potential of moringa leaf cream extract given topically to inhibit rat paw edema, the researchers hope to contribute information and insight to test the anti-inflammatory effect by inhibiting inflammatory mediators and the basis for research in humans (clinical trials).

CONCLUSION

This study concludes that 10%, 12%, and 14% of moringa leaves extract cream have anti-inflammatory effects. The moringa leaves extract 14% cream has better effectiveness than the concentration of 10% and 12%. The 14% moringa leaves extract cream inhibits rat paw edema as well as hydrocortisone 2.5% cream.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

ETHICS APPROVAL

This study had been ethically approved by the Ethics Commission of Faculty of Medicine Universitas Udayana with ethical clearance number 1737/UN14.2.2.VII.14/LT/2020.

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AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

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