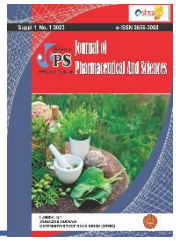
JOURNAL OF PHARMACEUTICAL AND SCIENCES
Electronic ISSN: 2656-3088
Homepage: https://www.journal-jps.com

ORIGINAL ARTICEL

JPS |Suppl 1 | No. 1 | 2023 |pp.276-282



Effect of giving ekor naga leaf extract gel (*Rhaphidophora pinnata*) on differential rat leukocytes induced by carrageenan air pouch

Pengaruh pemberian gel ekstrak daun ekor naga (*Rhaphidophora pinnata*) terhadap diferensial leukosit tikus yang diinduksi carrageenan air pouch

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ABSTRACT
Ekor naga leaves gel preparations have been pharmacologically proven to have an anti-inflammatory effect by reducing inflammation in the skin of mice. Leukocyte differential describes the repair of leukocyte cells in overcoming inflammation. The research aimed to determine the effect of administering ekor naga leaves extract gel on the differential number of leukocytes in mice induced by air pouch carrageenan. The research method used is experimental design. The treatment group consisted of 5 groups, with five mice in each group. Positive control group (Hydrocortisone®), Formula 0 (Gel Base), Formula 1 (Extract concentration 10%), Formula 2 (Extract concentration 15%), Formula 3 (Extract concentration 20%). The research data obtained was analyzed using one-way ANOVA with a confidence level of 95%. Research results show that Formula 2 is the best.

Keywords**:** *Gel, Rhaphidophora Pinnata, Leukocytes, Carrageenan*

ABSTRAK
Sediaan gel daun ekor naga telah teruji secara farmakologis memiliki efek antiinflamasi sebagai penurun radang pada kulit tikus. Diferensial leukosit merupakan gambaran dari perbaikan sel leukosit dalam mengatasi munculkan radang di dalam tubuh. Tujuan penelitian adalah untuk mengetahui pengaruh pemberian gel ekstrak daun ekor naga terhadap jumlah diferensial leukosit pada tikus yang diinduksi carrageenan air pouch. Metode penelitian yang digunakan adalah experimental design. Kelompok perlakuan terdiri dari 5 kelompok dengan jumlah tikus masing-masing kelompok 5 ekor. Kelompok kontrol positif (Hidrokortisone®), Formula 0 (Basis Gel), Formula 1 (Konsentrasi ekstrak 10%), Formula 2 (Konsentrasi Ekstrak 15%), Formula 3 (Konsentrasi ekstrak 20%). Data penelitian yang didapatkan di analisis menggunakan ANOVA satu arah dengan tingkat kepercayaan 95%. Hasil Penelitian menunjukkan bahwa formula 2 merupakan formula terbaik

Kata kunci*: Gel, Rhaphidophora Pinnata, Leukosit, Carrageenan*

INTRODUCTION

Pain is an unpleasant sensory and emotional condition related to tissue damage (Astika, Sani, & Elisma, 2022). Meanwhile, inflammation is a protective response that produces various inflammatory mediators in irritation, infection, or tissue injury. The inflammatory process involves many types of cells and mediators (Interleukin and THF-α), Reactive Oxygen Species, Prostaglandins, and Cyclooxygenase, which will have the effect of disrupting the microcirculation of blood proteins and other blood cells, which will trigger the emergence of an inflammatory response in the tissue in the form of vasodilation and increased vascular permeability (Alderton & Scanlon, 2021).

Ekor naga leaves are an herbal plant often used in the community, and preclinical testing data has been found to have pharmacological eff, acts including anticancer, antihyperglycemic, and anti-hyperuricemia. The effects that have been studied related to inflammation are the effects of ekor naga leaf extract as a wound healer and an anti-inflammatory agent (Anatasya, Sanik, & Muhaimin, 2021; Sani K, Rahman, Rahman, Samudra, & Floris, 2022).

Leukocytes are a type of cell that plays a role in the body's defense system, quickly responding to infectious or inflammatory conditions (Hettwer et al., 2022). It protects the body from various diseases with the phagocytic mechanism and produces antibodies. Differential leukocytes, such as neutrophils, eosinophils, monocytes, and lymphocytes, are markers of an inflammatory process in the body. The increased and decreased levels reflect white blood cells' responsiveness in preventing disease and inflammation agents, where the factors influencing the number are environmental conditions, age, and nutritional content in the body (Lubis, 2016).

One of the therapies for inflammatory conditions can be given through topical preparations. Topical drug administration has the advantage that it can work directly at the source of the disease without going through a long process to produce an effect, avoids first-pass metabolism, and is comfortable to use on the skin (Antunes-Ricardo, Gutierrez-Uribe, & Serna-Saldivar, 2015; de Oliveira et al., 2010). The gel is a type of topical preparation that is easy to make and provides a cool and comfortable feeling on the skin (Bokti & Saputri, 2018; Zamil, Zamil, Naser, & Kadhim, 2022).

Based on the problems above, the researchers tested the anti-inflammatory effect of ekor naga leaf extract gel in terms of the differential number of blood leukocytes in male white mice induced using the Carrageenan-Induced Air Pouch Method.

RESEARCH METHODS

Ekor naga leaves extract

Extraction was carried out using the maceration method using 70% ethanol solvent. Simplicia was macerated in 500 grams for three days, and the filtrate was obtained. Then remaceration was done twice to obtain a total of 6.3 Liter of macerate. The resulting macerate was concentrated using a rotary evaporator at a temperature of 50^0C.

Phenol Test

Weigh 0.5 grams of extract, add 10 mL of methanol, then react with 1-2 drops of FeCl3. Positive results if a dark blue-black color is formed.

Alkaloid Test

Weigh 0.5 grams of sample, then dissolve it in 5 mL of 2 N HCl. The solution obtained is divided in half, then Mayer's reagent is applied to tube one, and Dragendorf's reagent is applied to tube 2. A positive result is indicated by forming a white precipitate for the tube with Mayer's reagent and a light brown to yellow precipitate for the tube with Dragendorf's reagent.

Flavonoid Test

Weigh 0.5 grams of the extract in a test tube and add 96% ethanol. The mixture is shaken, heated in a water bath for 10 minutes, and filtered. The filtrate obtained was added with 0.2 grams of Mg powder and a few drops of concentrated HCl. An orange to reddish color indicates positive results.

Saponin Test

Add 0.5 grams of extract and 10 mL of hot water, and calm. Shake if foam is formed as high as 1-10 cm, which is stable for 10 minutes

and does not disappear with the addition of 2 N HCl, indicating positive saponin.

Tannin Test

Add 0.5 grams of extract, 1-2 drops of Fe Cl3, and gelatin. Positive results indicate a dark blue-black color.

Steroid and Terpenoid Test

Weigh 0.5 grams of extract, mix with 2 mL chloroform, and shake. Then, add a few drops of acetic acid and concentrated sulfuric acid each. Positive results for steroids form a blue-green ring, while the formation of a reddish-orange ring in the solution indicates positive results for terpenoids.

Ekor Naga Leaf Gel Formulation

Table 1. Design of Gel Preparation Formulations

Materials	Concentration%			
	F0	FI	FII	FIII
Ekor naga leaves extract	-	10	15	20
Carbopol	1,5	1,5	1,5	1,5
Glyserin	5	5	5	5
Propylen glykol	10	10	10	10
Trietganolamine	1	1	1	1
Methylparaben	0,1	0,1	0,1	0,1
Aquadest ad	100	100	100	100

Note:
F0: Formula that does not contain cinnamon leaf extract nanoparticles
FI: Formula contains 10% ekor naga leaf extract
FII: Formula contains 15% ekor naga leaf extract
FIII: Formula contains 20% ekor naga leaf extract

The gel is made by expanding carbopol with hot water 20 times, allowing it to swell and grind until thoroughly mixed. Methylparaben was dissolved in glycerin using a glass beaker. Grind the ekor naga leaf extract in a different mortar, add crushed propylene glycol until homogeneous, and add methylparaben dissolved in glycerin. Then, grind and add carbopol and triethanolamine until it forms a gel structure. The final stage is to add the remaining water until it reaches a weight of 100 grams and a gel mass is formed.

Induction of Inflammation

The test animals used in this research were male white rats of the Wistar strain. The treatment was divided into five treatment groups, namely positive control (Hydrocortisone 5%), negative control (Formula 0), ekor naga leaf extract 10% (Formula I), ekor naga leaf extract 15% (Formula II), ekor naga leaf extract 20% (Formula III). Before induction, the hair on the back of the test animal was shaved with a diameter of 3-5 cm. The fur is shaved using a razor and strengthened with Veet to help remove the hair on the rat's back properly. After 24 hours, the test animals were injected with 20 mL of air subcutaneously until an air sac formed on the rats' backs. Then, 24 hours later, 10 mL of air was injected using the subcutaneous method. On the third day, 4 mL of 2% carrageenan was injected. On days 4 to 8, 0.3 grams of gel is given twice daily. On the ninth day, the test animals were sacrificed by administering ketamine and xylazine anesthesia to facilitate blood collection, which would be used in the process of making blood smear preparations. Blood was drawn from the rat's heart and placed in a blood tube. The aim is to avoid errors in making blood smears so that more replications can be obtained in large quantities.

Differential Leukocyte Counting

Differential leukocyte calculations are carried out by making blood smear preparations. Making blood smear preparations begins with a blood sample of the mouse to be examined and attached to a glass object. Then, using the tip of another glass object, touch the blood drop at an angle of 450C and move it until a thin blood smear is formed. Then, leave the preparation to dry and fix the salaam for 5 minutes using a methanol solution. The stain used in this research was Giemsa dye. Giemsa is dripped on top of the preparation until the blood smear is covered and left for 30 minutes. Finally, the preparation was washed with distilled water and dried at room temperature. The leukocyte differentials observed were neutrophils, eosinophils, monocytes, and lymphocytes, counting up to 100 leukocytes found for each blood smear.

Data analysis

Data analysis used in this research used the descriptive method for extracting yield data

and phytochemical screening and one-way ANOVA methods with a confidence level of 95%, followed by Duncan's follow-up test.

RESULTS AND DISCUSSION

Ekor naga leaves are taken from the Mendalo Jambi outer city area. Determination carried out at Andalas University in Padang with Number 251/K-ID/ANDA/VI/2021 shows that the plant correctly identified is the ekor naga leaf of the Araceae family, Epiprennum pinnatum (L.) Engl species with the synonym Rhapidophora pinnata (L.f) Schoot.

The extraction method for ekor naga leaves the maceration method, which is carried out by soaking simplicia powder using an appropriate solvent. The advantage of this method is that the equipment used is simple and does not use heating, so the active compounds are still protected from damage due to heating. Five hundred grams of simplicia powder was macerated with 70% ethanol, resulting in a thick extract yield of 61 grams or around 12%.

Phytochemical screening is the initial process of screening components of secondary metabolite compounds that have pharmacological effects on the plant extracts. The results of phytochemical screening can be seen in Table 2.

Table 2. Phytochemical screening test results

	Information
Alkaloid	+
Flavonoid	+
Steroid	+
Saponin	+
Tanin	+
Fenol	+

Note: (+) = contains a group of compounds

Providing a topical preparation of ekor naga leaf extract gel has many advantages, including being easy to wash and an incredible feeling on the skin. Hence, it is comfortable to use, not sticky, does not require special treatment, and has good absorption into the skin so that optimization of drug absorption can be achieved. Sani et al. (2022) show that the ekor naga leaf gel preparation in Formula 2 is the most stable formula from the physical properties and cycling tests. The higher the concentration of the extract, the more runny the resulting gel preparation will be. This happens because the extract will have a cohesive force effect so that the bonds between carbopol molecules will also be reduced, and the resulting gel will be thinner. The physical form of the gel preparation can be seen in Figure 1.

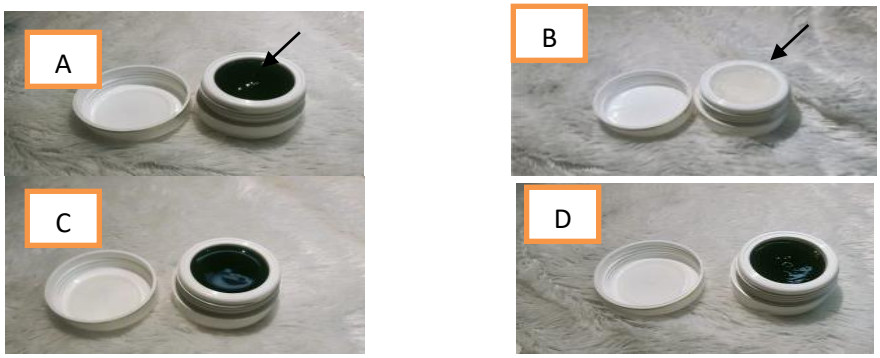

Figure 1. Ekor naga leaf ethanol extract gel (a) Gel base (b) F1 (10% extract) (c) F2 (15% extract) (d) F3 (20% extract).

This research has ethical clearance number 3097/UN 28.1.30/KL/2021, examined by the medical and health research ethics committee, Faculty of Medicine, Tadulako University. The anti-inflammatory test in this study used the method of forming air sacs on the backs of mice and then giving them carrageenan induction. This method can be used because it is easy to use and does not require special equipment to determine the percentage value of inflammation inhibition in test animals (Fehrenbacher & McCarson, 2021). Carrageenan is a mucopolysaccharide compound

from seaweed that plays a role in forming acute edema. Carrageenan will act as a foreign substance (antigen) which, if introduced into the body, will trigger the release of inflammatory mediators such as histamine, resulting in inflammation caused by antibodies that react with the antigen to counter its effects (Suryandari, Queljoe, & Datu, 2021).

Inflammation is a complex tissue reaction that occurs due to injury to cells. The inflammation used in this research is acute; inflammatory conditions can occur quickly. Increasing the number of pathogenic compounds in the body will increase leukocyte differentiation. Differential leukocytes are a group of leukocyte cell types based on cell size, granule color, and number of nuclei that can be detected by making blood smear preparations. Leukocytes are active in fighting infection or inflammation that occurs in the body by forming antibodies. The differential leukocyte results can be seen in Table 3.

Table 3. The average of leukocytes in the ekor naga leaves activity test

Formula	Average Number of Differential Leukocyte Cells			
	Stem Neutrophils	Segmented Neutrophils	Monocytes	Lymphocytes
Positive Control	16.20 ± 1.496[a]	15.00 ± 0.632[a]	1.40 ± 0.244[a]	77.20 ± 1.350[a]
F0 (Basis Gel)	34.20 ± 1.743[d]	37.20 ± 1,356[d]	5.00 ± 0.547[b]	52.00 ± 1.643[d]
F1 (Extract 10%)	34.00 ± 1.341[c]	29.40 ± 1.469[c]	4.40 ± 0.400[b]	59.40 ± 1.964[c]
F2 (Extract15%)	22.20 ± 1.200[b]	20.60 ± 0.812[b]	2.40 ± 0.244[a]	70.20 ± 2.870[c]
F3 (Extract 20%)	21.60 ± 1.435[b]	20.00 ± 0.632[b]	1.40 ± 0.244[a]	78.60 ± 1.691[a]

Notes :
a. The significance value was determined by one-way ANOVA analysis with a 95% confidence level.
b. Different lowercase superscripts on the same line indicated a significant difference (P <0.05).

Based on the data in the table above, it was found that there was an improvement in the values of stem neutrophils, segment neutrophils, monocytes, and lymphocytes. This is indicated by a significant difference when compared with the negative control ($p<0.05$). This shows that the chemical content of secondary metabolites in ekor naga leaf extract can improve the differential number of leukocytes in the blood, such as stem neutrophils, segment neutrophils, monocytes, and lymphocytes.

Neutrophils are a type of leukocyte cell that is quite often found in blood circulation. So when an inflammatory condition occurs, neutrophils are the cells that are most often released to the location where it occurs. Neutrophils have a size of about 14 µm. Under normal conditions, the percentage of neutrophils in male white mice ranges from 4.5% - 23.5%. There are two types of neutrophils, namely rod neutrophils and segment neutrophils. An increase in the number of neutrophils indicates an increase in macrophage collection activity at the site of infection. Neutrophils repair inflammation by extravasating from the vascular system to tissue by phagocytosis of antigens and collaborating with macrophages to release inflammatory mediators (Dillasamola et al., 2016).

Monocytes are granulocyte cells which account for around 3-8% of all leukocyte differentials. These cells have a diameter of 12-15µm. Monocytes result from the development of mononuclear phagocytes originating from the bone marrow. In inflammatory conditions, monocytes provide an immune response, including releasing inflammatory cytokines and presenting antigens to lymphocytes. When monocytes enter the tissue, they become macrophages and can survive for several months or years. Macrophages will play a role in angiogenesis and fibrosis. Macrophages themselves will be activated by various stimuli, such as microbial products that bind to Toll-Like Receptors (TLRs) and cytokines, one of which will

play a role in the production process of sensitized T Lymphocytes, Natural Killer Cells (NK cells) and other chemical mediators. Macrophages will work by eliminating various dangerous agents that arise from inflammation (Kratofil, Kubes, & Deniset, 2017).

Lymphocytes also have a fundamental role in the repair process when inflammation occurs, which is part of the adaptive immune response. In general, lymphocytes mediate specific immune reactions in fighting foreign molecules and recognizing them (memory function) to face the next attack. The interactions in the blood between neutrophils, monocytes, and lymphocytes illustrate the improvement in inflammation that occurs in test animals. This effect arises from the secondary metabolite chemical compounds in ekor naga leaf extract (Ramadhani et al., 2023).

This improvement in inflammation is supported by the chemical content of secondary metabolite compounds, namely alkaloids, flavonoids, steroids, saponins, and tannins. The mechanism of action of alkaloids is by suppressing the release of histamine by mast cells, thereby reducing the secretion of Il-1 by monocytes and PAF in platelets. Apart from that, alkaloids also play a role in activating glucocorticoid receptors to reduce the transcription process of genes involved in the inflammation that occurs. Flavonoids work by inhibiting eicosanoid-producing enzymes such as phospholipase A2, lipoxygenase, and cyclooxygenase so that they play a role in reducing the concentration of prostanoids and leukotrienes. Steroids also play a role in inhibiting the phospholipase enzyme and activating the glucocorticoid receptor. Furthermore, saponins work by inhibiting the release of pro-inflammatory substances stimulated by LPS, such as iNOS, IL, and INF-α, so that the formation of exudate due to inflammation and permeability of the vascular system can be prevented. Finally, tannin plays a role in supporting other compounds' work through the action of inhibiting the cyclooxygenase enzyme from prostaglandins (Astika, Sani, & Elisma, 2022).

The combined action of all secondary metabolite compounds contained in ekor naga leaf extract provides an optimal effect in curing inflammatory conditions in test animals if monitored from the leukocyte differential perspective. However, the results of evaluating the best gel formula preparation were formula two, which was physically stable. This result is also supported by statistical data regarding the leukocyte differential for formula two and formula three, which has almost the same effect, seen in the superscript letters indicating the same or not significantly different ($p<0.05$). So, the results of this study state that the best formula from this research is formula 2 in terms of the physical stability of the preparation and the optimal effect of the leukocyte differential.

CONCLUSION

Ekor naga leaf extract gel improves the number of differential types of leukocytes in the inflammatory condition of male white rats whereas Formula 2, with an extract concentration of 15%, is the best.

ACKNOWLEDGMENT

The article writing team would like to thank the Faculty of Medicine and Health Sciences (FKIK) Jambi University for providing research grants from the Faculty's PNBP funds with research contract number 371/UN21.11/Pt01.05/SPK/2021

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