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EFFECTIVENESS ANTI-INFLAMMATORY OF SUNGKAI LEAF (*Peronema canescens* Jack.) ETHANOL EXTRACT ON MALE RABBIT (*Oryctolagus cuniculus*)

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ABSTRACT

Sungkai leaves are medicinal plants used empirically by the people of Bengkulu, often found in the tropical rainforests of Bengkulu province. Sungkai leaf secondary metabolites include flavonoids, alkaloids, saponins, and steroids/triterpenoids. Flavonoid compounds have hundreds of bioactivities including antipyretic, analgesic, antidiabetic, anticholesterol and anti-inflammatory. The aim of this study was to obtain a new drug that has effectiveness as an anti-inflammatory from ethanol extract of Sungkai leaves (*Peronema canescens Jack)* n male rabbits (*Oryctolagus cuniculus)* The study used an experimental method using male rabbits as experimental animals. The samples were divided into 5, namely negative control, positive control, and 2 %, 4 %, and 6% bioplacenton and sungkai leaf extract. Each group was subjected to 2nd degree burns. Measurements were taken every day for 8 days after injury. The results of the study proved that there was a decrease in wound diameter from Sungkai leaf extract, on average at a concentration of 2% it decreased by 64% and at a concentration of 66% it decreased by an average of 13.3% and at a concentration of 6% it decreased by an average of 78,67 %. Based on the statistical results of the analisis of variance test, there is a significant difference.

Keywords: *Sungkai leaf extract, anti-inflammatory, Bioplacentone*

INTRODUCTION

Inflammation is a physiological process that responds to damage in a part of the body. Acute inflammation can be triggered by a variety of stimuli and is characterized by a rapid host response to sites of infection or tissue trauma, namely the delivery of leukocytes and plasma proteins, such as antibodies, to the site of inflammation. Chronic inflammation can last weeks, months, or even years after the acute inflammatory process (de Lima Souza *et al*., 2012).

Several chemical mediators are released during acute and chronic inflammatory processes. A large number of inflammatory mediators are released via the arachidonic acid pathway, including prostaglandins, as a result of the breakdown of arachidonic acid by cyclooxygenase enzymes. To overcome this, anti-inflammatory drugs are used, with a number of side effects related to the use of these drugs. Some of the side effects that stand out from the use of anti-inflammatory drugs are side effects on the gastrointestinal system, which increase the risk of stomach ulcers, as well as the cardiovascular system, which is at risk of increasing the occurrence of blood vessel blockage due to blood clots (Pountos *et al*., 2011).

Indonesia is known for its biodiversity, including rich medicinal plants. Indonesia is ethnically and culturally diverse. Each ethnic group has a different cultural treasure. Each ethnic group has a local wisdom, including the use of plants in traditional medicine. Knowledge about the use of medicinal plants by local indigenous ethnicities is very important for the development of traditional and modern medicine because many plant

extracts for modern medicine are found through local knowledge approaches. The utilization of data on medicinal plants resulting from ethnobotanical research is an effective way to discover new chemicals that are useful for medicine Utilization of data on medicinal plants resulting from ethnobotanical research is an effective way of discovering new chemicals that are useful for medicine (Kementerian Kesehatan RI, 2016).

One medicinal plant thought to have anti-inflammatory activity is the Sungkai plant (*Peronema canescens* Jack.) or the Sebrang teak plant, which belongs to the family Verbenaceae. Peronema Canescens Jack commonly found in Bengkulu Province, namely in tropical rain forests, in gardens or in the yard of the house which is planted as a fence. Sungkai plants can be used as mouthwashes for toothaches, medicine for external wounds, and medicine for internal wounds. The decoction of Sungkai leaves (*Peronema canescens* Jack.) is consumed by the people of Curup, Bengkulu Province, as a medicine for malaria (Badiaraja, 2014). Research that has been done previously found that there are several secondary metabolite compounds in the Sungkai plant (*Peronema canescens* Jack.), namely flavonoids, alkaloids, saponins, tannins, steroids and phenolics (Latief *et al*., 2021)

This study intends to obtain a new drug that has effectiveness as an anti-inflammatory from the ethanol extract of Sungkai leaves (*Peronema canescens* Jack) gainst male rabbits (*Oryctolagus cuniculus*).

RESEARCH METHODS

The laboratory experiments were conducted at the Pharmacology Laboratory of D3 Pharmacy, Faculty of Mathematics and Natural Sciences, University of Bengkulu. There was a treatment as well as a control group in male rabbit test animals by randomization with the Control Group Post Test Design research design (Notoatmodjo, 2002). The Ethics Committee Approval stage Number:1702/UN25.8/KEPK/DL/2022 from the Ethical Committee of the Medical Research Faculty of Dentistry University of Jember. Plant taxonomy was verified at the Biology Laboratory of the University of Bengkulu (number 358/UN30.28).LAB.BIOLOI/AM/2022

Equipment and Materials

The tools that will be used in this study are measuring cups (pyrex), beaker glass (pyrex), stirring rods, pipettes, razors, scissors, filters, analytical scales (Kern: ABS), hot plates (C-MAG HS 7) , gauze, tissue, ruler, bunsen, heat induction metal, shaver, Hot plate.
Materials: Sungkai leaves *(Peronema canescens* Jack.) from Bengkulu Tengah district, Aquadest, 96% ethanol, and Bioplacenton. The test animals used in this study were male rabbits aged 3 months with a body weight of 2000-2500 grams.

Research Procedure

Sampling of Sungkai *(Peronema canescens* Jack) Taken from Central Bengkulu district, the leaves selected are young and fresh leaves. Processing of leaf samples is picked as much as 500 grams then sorted dry (cleaned of dirt) and wet sorted (washed with running water), then dried without being exposed to direct sunlight. After that, the dried samples are then powdered ready for extraction. Sample Extraction The sample is weighed as much as 500 grams, then put into the maceration container. The 96% ethanol solvent is poured slowly into the maceration container containing the sample while stirring until the solvent is evenly distributed. The ethanol solvent was left up to 1 cm above the surface of the sample, and extraction was carried out for 3 × 24 hours and every 24 h. The ethanol was replaced with occasional stirring, and the filtered filtrate was evaporated using a Rotary Evaporator until a thick extract was obtained and dried. Na-CMC 1%. As much as 1 gram of Na-CMC was added gradually into a 100 ml beaker containing 50 ml of distilled water (70 °C) while stirring with a stir bar until a colloidal solution was formed, which was then made up to 100 ml. Preparation of Test Animals The test animals used were male rabbits *(Oryctolagus cuniculus)*, body weight 2000-2500 grams, 4-8 months old. The number of male rabbits *(Oryctolagus cuniculus)* used was 15 which were divided into 5 groups with each group

consisting of 3 rabbits. During the process, it is maintained that the needs for food and drinks are met. Male rabbits were fasted for 8 hours before treatment, but drinking water was still given *(ad libitum)* (Parveen *et al*., 2007).

Rabbits are shaved on the back area with a size of ± 2 x 2 cm using a hair clipper until the skin is clean, smooth, and free of hair or fur. Before injury, the rabbit was anesthetized, and then the shaved back area was injured with a coin that had been heated over a Bunsen for 5 minutes. The burns that occurred when hot coins were held for 5 seconds on the back of the rabbit in this study were superficial second-degree burns that appeared with damage to the skin tissue, followed by bursting of blisters. Wet the burn with a towel or cloth moistened with cold water for a few seconds. Each group consisted of 3 rabbits, and the backs of the rabbits, which had been shaved until smooth, and coins affixed to cause inflammation, were given bioplacentons (a positive control). Bioplacentons are drugs that contain placental extract and neomycin sulfate. This drug is commonly used to treat burns, infections, chronic wounds, and other types of wounds. Placenta extract triggers the formation of new tissue and wound healing, whereas neomycin works by preventing or treating gram-negative bacterial infections in the wound area. Na CMC 1% (negative control) and sungkai leaf extract (Peronema canescens Jack) 2%, 4%, 6% (treatment). In Phase II, the test animals were shaved until smooth, and coins affixed so that inflammation occurred were given bioplacenton (positive control), 1% Na CMC (negative control), and sungkai extract (*Peronema canescens* Jack .) 2%, 4%, 6% (treatment). Each group of experimental animals was topically treated with a dose of 3 × 1 per day using sterile gauze. The treatment was carried out for 8 days while being observed every day before and after treatment with the healing parameters, namely, the reduction in the diameter of the burn *(proliferation phase)*.

Data Analysis

Data is collected from observations with analysis by testing statistical analysis of variance.

RESULTS AND DISCUSSION

Results The maceration extraction method was used in this study. The maceration method is a cold method (extraction process without heating) and is suitable for samples with a soft texture. In addition, heating can damage the chemical components of simplicia. This method has the advantage that all parts of the simplicia are in contact with the solution. Maceration was performed by soaking 250 g of Simplisia in 96% ethanol. Ethanol is used as a solvent because of its ability to attract polar and nonpolar compounds. In addition, ethanol was chosen as a solvent because it is very effective in producing optimal levels of active ingredients, and fungi and bacteria cannot grow in more than 20% ethanol and are not toxic. Measurement of rabbit back burns treated with topical administration of the test preparation and the control group (cm):

Table I. Measuring results for the average decrease in the diameter of rabbit wounds

Treatment Group	Wound size H-0 to H-8 (in cm)									%
	H0	H1	H2	H3	H4	H5	H6	H7	H8	
Negative Control	1,5	1,5	1,5	1,5	1,46	1.37	1.23	1.07	1.02	32,00%
Positive Control	1,5	1,5	1,37	1,27	1,06	0.93	0.77	0.57	0.27	82,00%
SLE 2%	1,5	1,5	1,4	1,39	1,28	1,12	0,83	0,67	0,53	64,00%
SLE 4%	1,5	1,5	1,4	1,33	1,17	0,97	0,76	0,57	0,5	66,00%
SLE 6%	1,5	1,5	1,35	1,33	1,15	0,89	0,68	0,45	0,32	78,67%

Information : SLE is Sungkai Leaf Extract

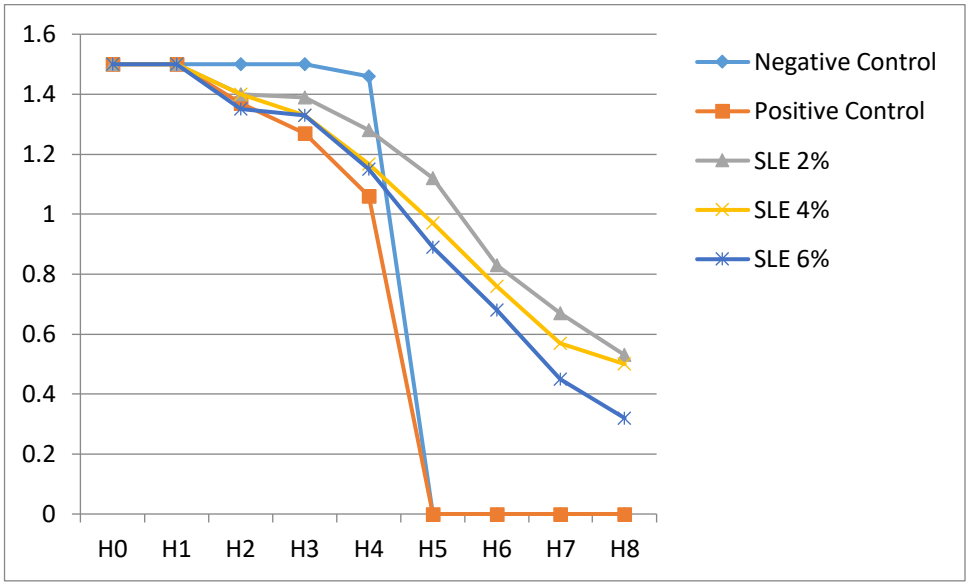

Figure 1. Graph of the average result of burn area in rabbits

Based on the results of this study, it was found that the administration of Na-CMC solution did not decrease the diameter of rabbit burns. This is because Na-CMC only serves as a negative control for the removal of inflammatory mediators in rabbit wounds that occur naturally.

In the administration of Sungkai leaf ethanol extract 2%, 4% and 6%, the average reduction in burn diameter was 64%, 66% and 78.67% on the 8th day produced. This is due to the possibility of the presence of flavanoid compounds contained in sungkai leaves which play an important role in the inhibition of prostaglandins (PGE) and lipooxigenase (LOX). and inhibit arachidonic acid metabolism and lysosomal enzyme secretion from neutrophil cells and endothelial cells. Inhibition of the cyclooxygenase pathway can have a wider effect because the cyclooxygenase reaction is the first step in the pathway leading to eicosanoid hormones such as prostaglandins and thromboxane. Inflammation that occurs in burns is due to a defense mechanism that the body uses to fight foreign substances that enter the body is not only caused by tissue damage due to trauma, chemicals, heat, or other phenomena (Guyton & Hall, 1997).

Symptoms of an inflammatory response include redness (rubor), which first appears over the inflamed area. When an inflammatory response occurs, the arteries that supply blood to the area widen, allowing more blood to flow into the local microcirculation. Blood vessels that were previously empty sometimes quickly widened and were filled with blood. Heat (color) occurs together with redness caused by an inflammatory response. Heat is an inflammatory reaction that occurs only on the surface of the body, namely, the skin. "The inflamed area of the skin becomes hotter compared to the surrounding area because the blood has a temperature of 37°C which spreads over the surface of the inflamed area of the body and spreads more than the normal area. increased permeability of capillary walls and migration of fluid and cells from the bloodstream into damaged tissues. With inflammation, leukocytes and proteins, especially albumin, more easily penetrate the capillary walls, followed by larger molecules; therefore, there is more protein than usual in the plasma tissue, which then leaves the capillaries and enters the tissue, creating tissue. Functional changes (functiono laesa) result from the inflammatory process (Price & Wilson, 2005). Movement in inflamed areas, both consciously and reflexively, is inhibited by severe physical pain and swelling, resulting in reduced movement (Nugroho, 2012).

In this study, there was a decrease in the diameter of the burn wound that was treated with Sungkai leaf extract, indicating that the extract has anti-inflammatory effects.

The anti-inflammatory effects depend on the concentration of the extract. namely, mediators of inflammation (Thamrin, 2020). Flavonoids have more than one hundred bioactivities. The bioactivities presented include antipyretic, analgesic, and anti-inflammatory effects (Wijayakusuma, 2011).

In the comparison group (bioplacentons), the average reduction in burn diameter was 82% on day 8. contained neomycin and placental extracts. Neomycin is used to prevent and treat bacterial infections. Placenta extract is a synthetic substance that resembles the human placenta. Its function is to trigger the formation of new tissue in injured skin and maintain skin elasticity and youthfulness (Robinson & Kosasih Padmawinata, 1995).

The results of the statistical analysis of the data showed a significant value, which means that there was a difference in the effect between the treatments, so it was said that there was an effect of giving Sungkai leaf extract on the anti-inflammatory effect of burns in male rabbits. at the time of reading the scale.

CONCLUSION

Based on the results of the research and testing the statistical analysis of variance method, it can be concluded that the ethanol extract of sungkai leaves *(Peronema canescens* Jack.) 2 %, 4% and 6% had an anti-inflammatory effect on male rabbits (*Oryctolagus cuniculus).*

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