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Research Article

Application of Purple Leaf Extract (Graptophyllum Pictum) In Wound Healing Process of Collagen Density

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ABSTRACT

Background: Purple leaves are derived from the Graptophyllum pictum plant, the Acanthacea family contains: flavonoids, saponins, and tannins. Purple leaf extract has anti-inflammatory and analgesic effects.
Purpose: This study observed an increase in the density of collagen in wounds in wistar rats.
Methods: The research subjects were 24 adult and healthy Wistar (Rattus norvegicus) mice, aged 3 months, with a body weight of 200 grams, which were divided into 6 groups of 4 rats each. The making of purple leaf extract is as follows, purple leaf powder weighed as much as 500 grams, then moistened with 96% ethanol as much as 500 ml. Research data were analyzed using the Kruskall Wallis and Mann Whitney tests.
Results: Statistical data analysis using the Kruskall Wallis test showed significant differences in collagen density ($P<0.005$).
Conclusion: Giving a 15% purple leaf extract gel increases collagen density.

Keywords: Purple leaf (Graptophyllum pictum), wound healing, incision, collagen.

INTRODUCTION

Gingivectomy is a gingival surgery that is often performed. Gingivectomy is done by cutting the gingiva in patients with gingival hyperplasia. It causes post-surgical injury so that periodontal dressing is given to close the wound[1]. One of the main components in the wound healing phase is collagen[2,3]. Collagen is the most protein in body tissue, including skin. Collagen has the ability, among others, in hemostasis[4], to interact with platelets, interact with fibronectin, increase fluid exudation, increase cellular components, increase growth factors and promote fibroplasia and increase epidermal proliferation[5].

The development of natural treatment technologies in herbal plants is increasingly becoming a concern[6,7]. Herbal plants are very rich in chemical compounds that are useful to help overcome human health problems. One of which is Graptophyllum pictum or known as purple leaves[8]. In traditional medicine, purple leaves are used for the treatment of wounds, swelling, ulcers, ulcers, and skin diseases[9,10]. Experimental extracts of purple leaves are effective in inhibiting swelling and decreasing vascular permeability[11].

Purple leaves are derived from the Graptophyllum pictum plant, the Acanthacea family contains: flavonoids, saponins, and tannins[12]. Purple leaf extract has anti-inflammatory and analgesic effects. There are phytochemical screening tests that indicate the presence of flavonoids in the ethanol extract of purple leaves, which acts as an anti-inflammatory by inhibiting. COX-2 further inhibits the formation of prostaglandin E2 in order to prevent prolonged inflammation and stop inflammatory response to swelling[11][11,13].

In this study, an incision was made on the back of the rat as an animal model. The application of purple leaf extract in this study was in the form of gel because the gel had faster absorption. Purple leaf extract gel is expected to be useful in wound healing, especially in the oral cavity. The treatment was carried out on the third, seventh and 14th days because this study was a study that observed wound healing components, one of which is collagen. The day was chosen because collagen appeared from day 3 and began to increase on days 7 and 14. Previous studies have shown that administration of purple leaf extract to wistar rats is not toxic[14]. Concentration of purple leaf extract 15 percent in

the form of ointments can accelerate wound healing by increasing fibroblast proliferation[15].

Based on the above description, it is necessary to conduct research on the administration of 15 percent purple leaf extract in gel form to wistar rat cut wounds against collagen density. This study aims to prove that topical application of purple leaf extract (Graptophyllum pictum) can increase collagen density in the wistar rat (Rattus norvegicus) wound.

MATERIAL AND METHOD

This research have been approved by the research team with number 292 / HRECC.FODM / XI / 2018. The research subjects were 24 adult and healthy Wistar (Rattus norvegicus) mice, aged 3 months, with a body weight of 200 grams, which were divided into 6 groups of 4 rats each. The three control groups were the groups that were not given purple leaf extract on the 3rd, 7th, and 14th days. Three treatment groups were groups that were given purple leaf extract on the 3rd, 7th, and 14th days.

The making of purple leaf extract is as follows, purple leaf powder weighed as much as 500 grams, then moistened with 96% ethanol as much as 500 ml. Purple leaf powder was put into a jar, flattened and added 96% ethanol as much as 2.5 l until submerged. The jar is tightly closed and stirred at 50 rpm for 24 hours. The results of maceration of purple leaf extract were filtered with a cloth filter and evaporated using a rotary vacuum evaporator to ethanol content of 0% for 4 hours. The results of maceration of purple leaf extract then evaporated / evaporated again on waterbath for 2 hours and obtained thick purple leaf extract with a concentration of 100% as much as 50 ml.

Making 15% purple leaf extract gel by mixing 3 gram carboxymethyl cellulose sodium (CMC Na) with warm 100 ml distilled water little by little, stirring using mortar and pastle until homogeneous for 15-20 minutes. Next, 85 grams of CMC Na gel was mixed with 15 grams of purple leaf extract, stirred until homogeneous using mortar and pastle for 2-4 minutes.

24 wistar rats were anesthetized using ketamine 0.1 mg / kg body weight by intramuscular injection in the groin, then 2 cm long and 2 mm deep using sterile handles and scalpels until fascia was seen in each animal. The application of purple leaf extract gel was carried out using a syringe in the wistar rat wound of the treatment group while in the control group, the incision wound was left without any treatment. In the treatment group, purple leaf extract gel was given to the incision topically using a 0.5 ml syringe once per day every 11am.

The skin of the rat's back that has been made incision, cut by 2cm x 2cm using a handle and scalpel. Rat's back skin tissue was put into a plastic pot containing 10% formalin phosphate buffer solution, then proceed with making preparations and painting using Masson's Trichome (MT).

Collagen density that has been observed is then included in the score criteria that have been determined as follows[16]:

0 = Lack of collagen fibers present

1 = Low collagen density, collagen fibers appear thin or very little

2 = Medium collagen density, visible collagen fibers spread thin

3 = Dense collagen density, thick collagen fibers spread

4 = The density of collagen is very dense, thick collagen fibers collect

Data normality test with Kolmogorov Smirnov test, statistical analysis test with Kruskall Wallis test and Mann Whitney test.

RESULT

The results of research conducted on 24 wistar rats on the application of purple leaf extract in the wound healing process of collagen density. In the control group no gel was given, in the treatment group the 3rd, 7th, and 14th days were given purple leaf extract gel with a concentration of 15%.

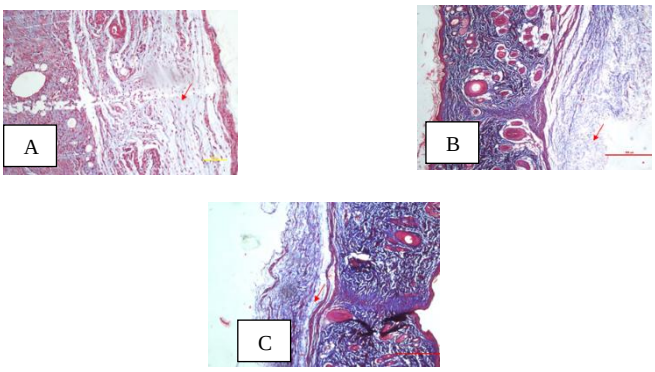

Fig.1: Density of control group collagen (A) day 3, (B) day 7, (C) day 14

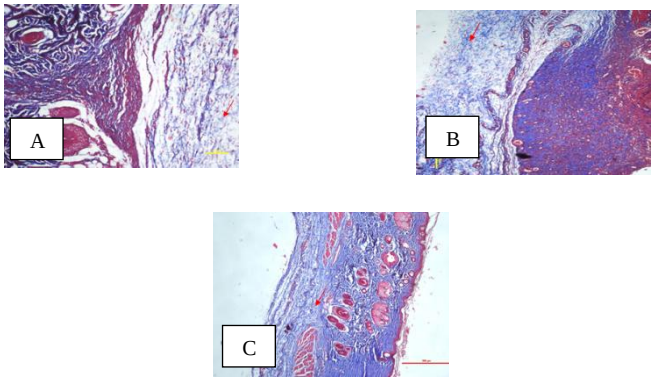

Fig.2: Treatment group collagen density (A) day 3, (B) day 7, (C) day 14

The data in each sample is quantitative data, where the average collagen density in each group can be seen in table 1. The average collagen density is calculated by dividing the collagen connective tissue area in the preparation into three fields. All of these examinations use a Nikon ECLIPSE E200 electronic microscope equipped with a calibrated micrometer. In the incision post-incision wound on the 3rd, 7th, and 14th day, preparations were made by MT painting. Observation conducted by looking at collagen density scoring in preparations using a 400x magnification electronic microscope. The results of collagen density calculation in preparations can be seen in table 1 below:

Table 1: Collagen Density Mean and Standar Deviation of each Group.

Group	n	Mean	Standard Deviation
K1	4	2,25	0,957
P1	4	3,00	0,816
K2	4	2,50	0,577
P2	4	4,00	0,000
K3	4	2,75	0,500
P3	4	4,00	0,000

Table 2: Mann Whitney Analysis result

	K1	P1	K2	P2	K3	P3
K1		0,278	0,752	0,013*	0,405	0,013*
P1			0,343	0,046*	0,617	0,046*
K2				0,013*	0,495	0,013*
P2					0,011*	1
K3						0,011*
P3						

(*) = Significant

From the results of the Mann Whitney test (table 2), there are significant differences between groups, namely between the 3rd day control and the 7th day treatment, the 3rd day treatment group with the 7th day treatment group, the 7th control group with the treatment group day 7, the control group day 14 with the treatment group day 7, the control group day 3 with the treatment group day 14, the treatment group day 3 with the treatment group day 14, the control group day 7 with the treatment group day 14, and the control group day 14 with the treatment group day 14.

DISCUSSION

This research was carried out by making incisions on the backs of mice as experimental animals. Rats were used as experimental animals in this study because mice have the same healing mechanism as humans. Only mice have faster healing time than humans[17]. The study uses 15 percent purple leaf extract in gel preparations to observe the comparison of the amount of collagen density on the third, seventh, and 14th days in the wound healing process. Gel preparations have faster absorption ability. According to previous studies, the concentration of purple leaf extract 15% in the form of ointments can accelerate wound healing by increasing fibroblast proliferation[15].

In histological observation, when compared between the control group in the third, seventh, and 14th day, it showed negligible differences in collagen density. Because no purple leaf extract was given so it did not accelerate wound healing. This can be interpreted as the administration of purple leaf extract affects the speed of wound healing. In incisions that are not given, purple leaf extract (control group) can activate protein kinases that phosphorylate kappa-B inhibitors (Ikb) and will cause Ikb degradation, resulting in translocation of Nuclear Factor kappa B (NF-kB) to the nucleus. NF-kB binds to the target gene and then stimulates transcription of inflammatory mediators so that TNF-α increases[18]. Uncontrolled pro-inflammation will cause chronic inflammation which can slow wound healing.

The observations in the 3rd day control group compared with the 3rd day treatment group did not show significant differences in collagen density. This is likely to occur because the administration of purple leaf extract has not been effective in increasing collagen on the 3rd day. Researchers report that new collagen fibers will be secreted by fibroblasts on the 3rd day after the onset of injury[19].

The observations in the treatment group on day 3 compared to days 7 and 14 showed a significant difference in collagen density. The observations between the control group were compared with the treatment group, namely the control group on the 3rd day and the treatment on the 7th day, the control group on the 7th day and the treatment on the 7th day, the control group on the 14th day and the treatment on the 7th day, control group day 3 and treatment day 14, control group day 7 and treatment day 14, control group day 14 and treatment day 14 showed a significant difference in collagen density. This is due to the presence of flavonoids in purple leaves as anti-inflammatory[18]. Flavonoids inhibit Ikb degradation by protein kinase, so that NF-kB translocation to the nucleus is inhibited, NF-kB in the nucleus decreases resulting in transcription of TNF-α inflammatory mediators and TNF-α production decreases, so inflammation is well controlled[20]. Reduction in TNF-α causing macrophages to secrete growth factors, namely FGF, PDGF, TGF-β, and EGF, these growth factors induce fibroblast proliferation so that fibroblast proliferation increases and collagen synthesis by fibroblasts increases[21].

CONCLUSION

Giving a 15% purple leaf extract gel increases collagen density.

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