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Hispathology And Effectiveness Of Wound Healing Cream Karo Oil Herbal Extract On Male Mice In North Sumatra, Indonesia

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ABSTRACT

Karo oil is one of traditional medicine originating from North Sumatra. Karo oil has many properties including accelerating wounds healing on the skin. Karo oil is a traditional medicine in the form of oil so that it is impractical to be used because of its sticky so that a cream form is made which is more practical to be used. This study aims at determining the effectiveness of wound healing in cream preparations from the plant n-hexane extract used in the manufacture of karo oil and tissue repair in the incision observed from its hyspatology. This study used an experimental method, used test materials derived from plants which were the ingredients for making karo oil. The extract was obtained by maceration using n-hexane pa solvent. The stages of this research included the manufacture of plant n hexane extract as raw material for karo oil and formulating it into cream preparations in several concentrations, namely 2.5%, 5%, and 9%. The results shows that the cream preparation made from the karo oil plant had the effect of wound healing with the best concentration of 9% in wound healing.

Keywords : cream; skin; wound; karo oil

INTRODUCTION

The prospect of developing medicinal plant production is increasingly fast considering the development of modern and traditional medicine industries is increasing.This condition is also influenced by the increasing public awareness about the benefits of plants as medicine, people are increasingly aware of back to nature by consuming naturalmedicines. Many people have to

improve their health status by using or consuming natural products. Although modern medicine is developing rapidly, the potential for traditional medicine, especially those derived from plantsremains highly. It is since traditional medicines can be obtained without a doctor's prescription, can be mixed by themselves, the raw materials do not need to be imported and plants can be grown by the users themselves (Sari, 2012; Nasution et al., 2020).

In Indonesia there are still many people who use plants in their daily life, especially those who live around the forest. Indonesia, like other Southeast Asian regions, has a high potential for traditional medicinal use of plants. The use of plants as traditional medicine is also increasingin demand by the community because it has been proven that medicines derived from plants are healthier and without causing any side effects when compared to medicines derived from chemicals. However, the problem for traditional medicine enthusiasts is the lack of adequate knowledge and information about various types of plants commonly used as the ingredients for traditional medicines and how they are used (Sembiring et al, 2013).

The use of plants by certain ethnicities/tribes is called ethnobotany. Ethnobotany studies do not only collect useful plants, note local names and how they are used. In the framework of theoretical interest, ethnobotany needs to be expanded with an interdisciplinary approach between botanical science and social science. Emphasis that is carried out in an interdisciplinary manner will be able to solve problems that include socio-cultural aspects and people's perceptions and understanding of plants and their use in the life of a community group. Ethnobotany studies will reveal the way of thinking of a community group, concepts regarding plants, policies in cultivation use, and conservation of biodiversity, which have traditionally been shrouded in cultural rules and values, beliefs and rituals (Nasution, 2009; Aziz et al., 2019).

One of the famous traditional medicine in North Sumatra is treatment using karo oil. Treatment using karo oil has been carried out from generation to generation. Using and utilizing plants as health support materials is one form of application of Karo community understanding in the management of natural resources. Based on research (Nasution et al, 2020) it was obtained that 42 types of karo oil ingredients contained in the plant parts used are leaves, rhizomes, roots, stems, seeds, fruits, flowers and tubers. Many of the properties obtained from karo oil include healing external wounds, drying and treating burns, treating itching, treating rheumatism, warming the body and treating sprains. Karo oil is an external medicine which use is sufficiently applied, rubbed or massaged on the part of the body that hurts.

Karo oil has been widely known and used by the wider community, especially in North Sumatra, therefore research is needed to make a more practical cream preparation derived from the plant ingredients for making karo oil. Given the proliferation of foreign products that contain lots of chemical substances, even though the products belonging to the country themselves are very rich in benefits.

METHODOLOGY

This research used n-hexane extract from plant as raw material to make karo oil. This research was conducted at the Pharmacology Laboratory and Pharmacy Laboratory of the Faculty of

Pharmacy, Tjut Nyak Dhien University, Medan. The research was conducted from February-April 2019. This research was an experimental study. The plants used in the manufacture of karo oil were macerated with n hexane solvent and made in cream preparations and the effectiveness of the cream preparations was seen in the wound healing process. The animals used in this study were mice (Mus musculus) with an incision on their back skin. The number of samples used in this study was calculated based on the sample size formula according to Federer, namely 25 mice with 5 mice in each group. The data obtained were analyzed using SPSS. The stages of making hispatological preparations were carried out using samples that had been fixed in a 10% BNF solution inserted into a basketball tissue and labeled. Tissue samples were dehydrated with stratified alcohol (70, 80%, 90%, and 95%) and absolute alcohol (I, II) for 2 hours respectively. Next step was clearing, which was by inserting the sample into the silole (I, II and III) for 1 hour each. Afterwards, it was continued with infiltration in paraffin I, II, III at a temperature of 60^{o}C for 1 hour each. Then the sample was embedded (embedding) in paraffin and blocking tissue. The tissue blocks were sliced using a microtome with a thickness of 5µm and placed in a slide that had been coated with Entellan® adhesive.

RESULTS AND DISCUSSION

The results of phytochemical screening of n-hexane extract were carried out to obtain information on the group of compounds contained therein.

Table 1**.**Phytochemical Screening Data of Karo Oil n-hexane extract

No.	Compound group	Check up result
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Steroids	+

Information: (+) = Contains a compound; (-) = Does not contain compounds

Based on table 1, it showsthat the results of phytochemical screening of the n-hexane extract of the karo oil raw material give positive results (+), which contain alkaloid compounds, flavonoids, tannins, saponins, steroids. The mayer test showed a dissolved yellow color, Bouchardat formed a brown sediment, and Dragendorf showed a brick-red precipitate. The phytochemical test results of n-hexane karo oil against the positive flavonoid compound, from the tests carried out in the experiment showed the formation of an orange yellow color. The results of phytochemical screening of plant n-hexane extract as raw material for karo oil against positive glycoside compounds, from the tests carried out in the experiment showed that the formation of red-orange to purple colors. The results of the phytochemical screening of the karo oil n-hexane extract against the positive saponins group compounds, from the tests carried out on the experiment showed the formation of a steady foam for not less than 10 minutes at a height of 1-10 cm.

The average wound length after giving karo oil n-hexane on the 1st day , 7thday and 14thday can be seen in Table 2.

Table 2. Average data of wound length after administration of n-hexane cream extract, plant extract as raw material for karo oil on 1st day , 7th day and 14th day.

Treatment	Average wound length (cm)					
	1st Day	3rd Day	6thDay	9thDay	12thDay	14thDay
Negative Control	1.00	0.98	0.9	0.82	0.7	0.61
Betadine Cream	1.00	0.94	0.85	0.7	0.59	0.37
2.5% cream	1.00	0.95	0.87	0.79	0.63	0.58
5% cream	1.00	0.92	0.84	0.76	0.6	0.55
7.5% cream	1.00	0.89	0.81	0.7	0.57	0.42

The average wound length after giving n-hexane karo oil on on the 1st day , 7th day and 14th day can be seen in table 2.In Table 2, the lowest average length of the wound is found in the treatment of the betadine cream group with an average of 0.37 cm on the 14th day while the highest average of wound length is found in the control group treatment with an average of 0.61 cm, It is since the control group was treated without treatment.

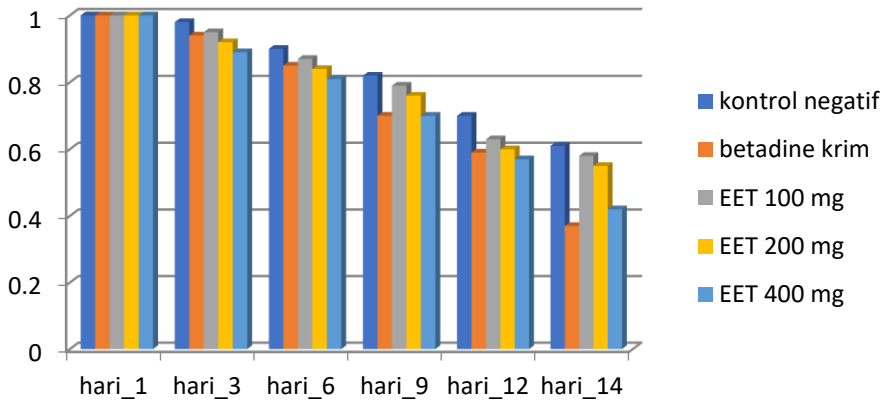

Figure 1. Graph of Average Reduction of Incision Length in Karo oil n-hexane cream preparations on the 1st,3rd,6th,9th,12th and 14thday.

Graph 1 above shows that the largest average reduction in wound length is in the betadine cream group on the 14th day, compared to other groups,since the content of each povidone iodine, where the mechanism of action is to inhibit bacterial infections caused by bacteria. According to (Paputungan, 2014) povidone iodine has the advantage of regulating moisture from wounds and providing a balanced moisture atmosphere.

The wound is the loss or destruction of some body tissue. This situation can be caused by trauma to sharp or blunt objects, changes in temperature, chemicals, explosions, electric shocks or animal bites (Liana& Utama, 2018). Wound healing was influenced by many factors including the type of drugs used. The use of medicines for wound healing can be done in various kinds and types, one of them is the use of natural traditional medicines. The wound healing process was heavily influenced by fibroblasts. The main process of fibroblast growth

occurred on the 7thday to 14th day. Fibroblasts are one of component of wound healing in the form of cells that were distributed widely in connective tissue.

In this treatment there was an improvement in the skin tissue due to the topical application of n-hexane karo oil extract. After phytochemical screening, it was found that karo oil contains alkaloids, flavonoids and saponins which can repair tissue. Based on the results of the study (Pusparani et al, 2016) alkaloid compounds were able to act as antibacterials to prevent bacterial infection wounds and disrupt the peptidoglycan constituent components in bacterial cells. Saponins worked by forming collagen and had the ability as a cleanser and antiseptic which kills and prevents the growth of microorganisms that arise in the wound so that the wound does not have severe infection.

The presence of secondary metabolites contain in karo oil leaf extract is associated with antibacterial activity. One of them is wound treatment which can prevent infection in wounds and accelerate wound healing. Based on (Hafizah et al, 2017) the best antibacterial activity was gram-positive compared to gram-negative bacteria. It is since there were differences in cell wall structure between gram-positive and negative bacteria. This antibacterial test was done to get the right antibacterial agent for the treatment of infectious diseases caused by bacteria.

Based on (Prasetyo et al, 2010) reepithelialization is a stage of wound repair which includes mobilization, migration, mitosis, and differentiation of epithelial cells. Wound healing was strongly influenced by reepithelialization, because the faster the reepithelialization process, the faster the wound will be closed, so the faster the wound will heal. The speed of wound healing can be affected by the substances contained in the drug given, if the drug had the ability to promote healing, then the growth of new cells stimulated the skin. Papaya leaves has the activity of accelerating the wound healing process in subjects of research by accelerating the reepithelialization process, increasing the formation of connective tissue on the skin so that it can be used as an alternative for wound healing.

Furthermore, the data were analyzed by using statistical tests. The statistical test was conducted to determine the significance of the effect of the treatment on the sample. Before the statistical test was carried out, a normality test was carried out to determine whether the data distribution was normal or not. After being tested, the normality value showed that the data distribution in the sample treatment group was normally distributed ($p > 0.05$). Then it was continued to Duncan test. The average data of Duncan's Post-Hoc average difference test on the healing of mice's incisions from the 1st day to the 15thday can be seen in table 3.

Table 3.Duncan Post-Hoc Mean Difference Test Data on the healing of mice incisions from the 1st day to the 14th day.

Treatment	N	Day				
		3	6	9	12	14
Negative Control	5	,98^{d}	,9^{c}	,82^{d}	,7^{d}	,61^{d}
Betadine Cream	5	,94^{b}	,85^{b}	,7^{a}	,59^{a}	,37^{a}
2.5% cream	5	,94.8^{c}	,87^{b}	,79^{c}	,63^{c}	,58^{c}
5% cream	5	,92.2^{b}	,84^{b}	,76^{b}	,6^{b}	,55^{c}
7.5% cream	5	,89^{a}	,81^{a}	,7^{a}	,57^{a}	,42^{b}

Note: Unequal letters superscript shows real difference ($p < 0.05$) ^{abcde}

Based on table 3 above it shows that on the 3rd day of Duncan Post-Hoc average difference test, there is a significant difference among betadine cream and negative control, 2.5% cream and 7.5% cream. However there was no significant difference between Betadine cream and 5% cream. On the 6th day of Duncan's Post-Hoc mean difference test, there was a significant difference between 7.5% cream and negative control. However there was no significant difference among betadine cream, 2.5% cream, and 5% cream. On the 9th day of Duncan's Post-Hoc mean difference test there was a significant difference between negative control with 2.5% b cream and 5% cream. However, there was no real difference between the betadine cream and 7.5% cream. On the 12thday of Duncan's Post-Hoc mean difference test, there was a significant difference between negative control with 2.5% b cream and 5% cream. However, there was no real difference between betadine cream and 7.5% cream. On the 15th day of Duncan's Post-Hoc mean difference test, there was a significant difference between negative control, betadine cream and 7.5% cream. But there was no real difference between 2.5% cream and 5% cream.

The results of the average number of fibroblasts on the wound healing of male mice (Mus musculus) on the 15th day can be seen in table 4.

Table4. Mean Score Data and SD Fibroblasts

Treatment	Mean Fibroblast Score ± SD
Negative Control	2.2 ± 0.45
Betadine Cream	5.6 ± 0.89
2.5% cream	4.0 ± 0.71
5% cream	11.4 ± 1.34
7.5% cream	13.4 ± 1.14

Based on table 4, it can be seen that there was an increasing in the mean score of the number of fibroblasts in all treatment groups. Treatment in the 7% cream group had the highest mean fibroblast score, namely 13.4 ± 1.14, and the lowest mean fibroblast score was in the negative control group, namely 2.2 ± 0.45. To prove the existence of differences between groups and an increase in the number of fibroblasts, then the results of the mean score for the number of fibroblasts were analyzed using Duncan test. In fibroblasts Duncan's post-hoc mean difference test there was a significant difference between negative controls for betadine cream, 2.5% cream, cream 5%, and cream 7.5%.

The results of the average score of collagen density on the wound healing of male mice (Mus musculus) on the 14thday can be seen in table 5.

Table 5. Data mean of Collagen Score and SD

Treatment	Mean ± SD of Collagen Score
Negative Control	1.8 ± 0.45
Betadine Cream	13.8 ± 1.10

2.5% cream	3,4 ± 1.14
5% cream	16.4 ± 0.55
7.5% cream	17.6 ± 0.55

Based on table 5 it shows that an increasing in the mean score of the amount of collagen are in all treatment groups. Treatment in the 7.5% cream group has the highest mean fibroblast score, 17.6 ± 0.55, and the lowest mean collagen score is in the negative control group, namely 1.8 ± 0.45. To prove the difference between groups and an increase in the amount of collagen, the mean score for the number of fibroblasts was analyzed using Duncan test. In Duncan Post-Hoc mean difference test fibroblast cells, there is a significant difference among negative controls for betadine cream, 2.5% cream, 5% cream, and 7.5% cream.

Microscopic picture of the density of collagen and fibroblast cells in various treatments can be seen in Figure 2.

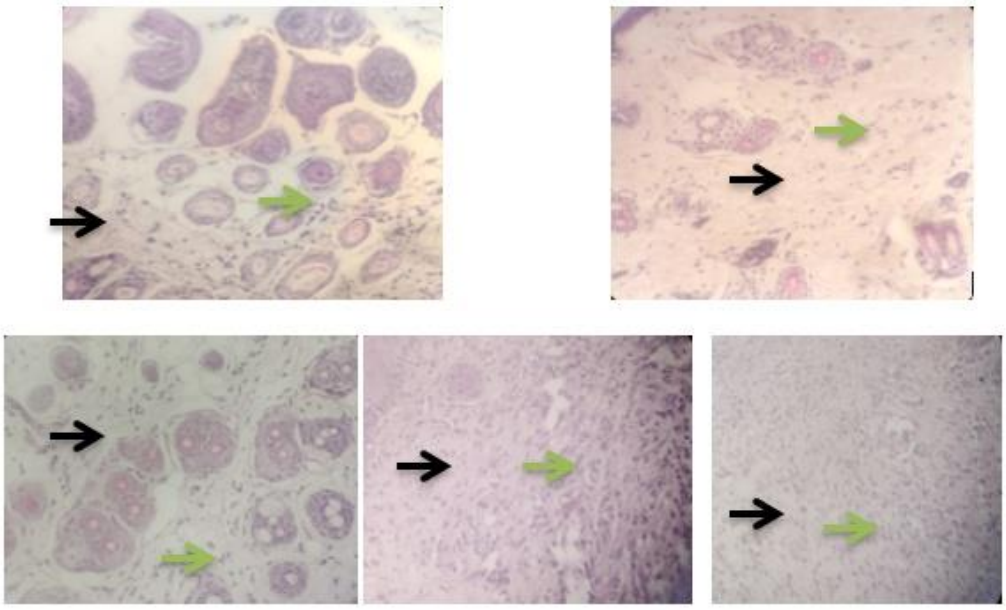

Figure 2.Microscopic picture of fibroblast cells and collagen density in various treatments. Magnification 400x. HE staining. (a) Control group, negative; (b) the betadine cream group (c) the cream group 2.5%; (d) 5% cream group; (e) 7.5% cream group. Fibroblasts () and collagen () cells.

In Figure 2 it shows that histopathological observation of the incision on the 14thday in the negative control group (a) the density of collagen fibers and fibroblasts was the lowest than the other groups and its spread is rare. In the betadine cream group (b) the density of collagen fibers was moderate and fibroblasts were low. In the cream group 2.5% (c) the density of collagen fibers and fibroblasts was low; In the cream group 5% (d) the density of collagen fibers and fibroblasts was tight. In the cream group 7.5% (e) the density of collagen fibers and fibroblasts was very tight.In all treatment groups there were visible injuries, indicated by the empty space in the pieces of mice's skin tissue. Swelling occured due to the induction of alloxan which had changed the balance of blood glucose levels. Based on (Wang et

al, 2017) the role of fibroblasts is very large in the repair process, which is responsible for the preparation of producing protein structure products that will be used during the tissue reconstruction process. The increasing in the number of fibroblasts was caused by flavonoid compounds. The proliferation of fibroblasts in the process of wound healing was stimulatednaturally by interleukin-Ib (IL-Ib), platelet derived growth factor (PDGF), and fibroblast growth factor (FGF).

Saponins can increase the proliferation of monocytes so that they can increase the number of macrophages. Macrophages will secrete the growth factor such as FGF, PDGF, TGF-β, and EGF which can attract more fibroblasts to the wound area and synthesize collagen and increase capillary blood vessel proliferation. Saponins contained in karo oil are useful for triggering the formation of collagen which plays a role in the wound healing process (Suharto & Etika, 2019).

The wound healing process was strongly influenced by the role of fibroblast migration and proliferation in the wound area. Proliferation of fibroblasts at the stage of healing the wound indicated a fast healing process. The main process of fibroblast growth occurred on the 7^{th} to 14^{th} day after injury and after that it was continued to improve until the skin structure wasback to normal.

At the beginning of healing, fibroblasts had a contractile ability called myofibroblasts, which caused the wound edges was pulled and then closed, so that the two wound edges sticked together. As healing progresses, the fibroblasts increased. These cells produced collagen, so the granulation tissue then collected the connective tissue matrix progressively, eventually resulting in dense fibrosis. The more connective tissue in the wound, the greater the power of wound contraction so that the side of the wound will be stretched and cause the wound to shrink (Amita, 2017).

Based on (Parampasi & Soemarno, 2013) the role of fibroblasts is very large in the repair process. Immediately after an injury occurred, fibroblasts movedactively from the tissue around the wound into the wound area, then they proliferated which played a role in reconstructing new tissue. Collagen fibers was formed on the edges of the wound, but initially these fibers were oriented vertically and did not bridge the wound. The proliferation of epithelial cells caused the epidermal layer thicken. Collagen fibers multiplied and began to bridge the wound. The proliferation phase would end when the epidermal epithelium and collagen layers have formed.

CONCLUSION

Based on the results of research that has been carried out on the n-hexane extract of karo oil, it is concluded that the n-hexane extract worked effectively in repairing incision wound tissue in mice as seen from the wound diameter and its hispatology.

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