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The Effect of the Treatment with Salve of Topical Tintir Castor Bark Extract (*Jatropha Multifida* L) on the Number of Fibroblast, Fibrin Formation, and Density of Collagen in the Wound Healing Process of the Rat with the Acute Injury Model

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Abstract---The use of traditional medicine in Indonesia is part of the culture of the nation and much utilized by society. This study aimed to determine the effect of the Treatment With Salve Of Topikal Tintir Castor Bark Extract (*Jatropha Multifida* L) on the Number of Fibroblast, Fibrin, Formation, And Density of Collagen In The Wound Healing Process of The Rat With The Acute Injury Model through excision diameter of 0.8 cm x 0.8 on the rat back. The research was conducted in the Animal laboratory of Hasanuddin University and the Education Animal Clinic (KHP), UNHAS. The method used in this research is the post-test control group design only. The sample consisted of 45 Wistar rats were divided into 3 groups: negative control (Vaseline), positive control (Oxycytetracilin 3%) and extract stem tintir distance. Data analysis using one way test anova. The study results indicated that on day 3 (tree) the fibroblast showed a significant difference between groups of negative group and the tintir castro stem group of 10% ($p=0.081$) and there was a significant difference between the positive group and the tintir castro stem group of 10% ($p=0.167$); and on day 7 (seven) and day 14, there was a difference but not significant. As for the fibrin there was no significant difference between the negative group and the tintir castro stem group of 10% ($p=0.139$) there was a difference of meaning between the positive group and the tintir castro stem group of 10% ($p=0.024$) while on day 7 and day 14 there is a difference but not significant. As for the collagen on the day 3 there was a significant difference between the negative group and the tintir castro stem group of 10% ($p=0.116$) there was a difference but not significantly between the positive group and the tintir castro stem group of 10% ($p=0.089$) and on day 7 and day 14 there was a difference but not meaningful. Thus, the 10% of the castro extract had no effect to an increase in the number of fibroblasts, but significantly reduce the formation of fibrin on the seventh day and increase density of collagen on day 14.

Keywords---acute wound, salve of tintir castro stem bark extract, fibroblasts, fibrin, collagen.

Introduction

The incidence of injuries is increasing day by day along with the complexity of our daily activities. In carrying out activities, we often experience accidents that result in injuries, both minor and serious injuries, and injuries can be preceded by trauma. Wounds are damage or loss of body tissue that occurs due to a factor that interferes with the body's protective system (Velmar et al., 2009). These factors include trauma, temperature changes, chemicals, explosions, electric shocks, or animal bites. The shape of the wound varies depending on the cause, there are open and closed. An example of an open wound is an incision where there is a linear tear in the skin and underlying tissue. One example of a closed wound is a hematoma where a broken blood vessel causes blood to collect under the skin (Pusponegoro, 2005).

The body has a physiological response to wounds, namely the wound healing process. The wound healing process consists of various complex processes to restore tissue integrity. During this process, blood clots, acute and chronic inflammatory responses, neovascularization, cell proliferation and apoptosis occur. This process is mediated by various cells, cytokines, matrix, and growth factors (Falanga, 2007). Dysregulation of this process can lead to complications or wound abnormalities, namely hypertrophic wounds and keloids. Healing of skin wounds without outside help goes naturally but sometimes special handling of the wound is needed to help the process. Therefore, it is important to understand the wound healing process (Eslami et al., 2009).

The wound healing process is an attempt by the tissue to undergo injury to restore normal function and structural integrity after trauma (Leong & Philips, 2012). Wound healing process takes place in all tissues and organs of the body. Healing is a complex process involving coordinated interactions between various immunological and biological systems involving several overlapping steps including inflammation, granulation tissue formation, re-epithelialization, matrix formation and remodeling (Hanstan et al., 2008).

One of the plants used as traditional medicine is tintir distance (*Jatropha multifida* L.). There are various uses of the *Jatropha* tree in everyday life, including the sap on the tree can be used to treat new and swollen wounds by applying the sap found on the leaves of dang stems on the wound (Hasriana, 2008). *Jatropha tintir* (*Jatropha multifida* L.) contains compounds that can be used as drugs including phenolic compounds, flavonoids, saponins and alkaloid compounds (Ehsanet et al., 2011). Flavonoids function as antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane (Fawcet, 2002).

Research conducted by Sillmar et al (2014) to test the antibacterial properties of *Jatropha tintir* (*Jatropha multifida* L.) using ethyl acetate extract and methanol extract found that ethyl acetate extract was more effective as antibacterial than methanol extract. Research has also been conducted by Sillmar et al (2014), to see the difference in the effect of wound care in guinea pigs using tintir distance resin compared to using 10% povidone iodine. days compared to those using 10% povidone iodine 7 -8 days.

Parameters commonly used to assess the wound healing process are molecular parameters including Fibrin, fibroblast, and collagen. Platelets degranulate releasing alpha granules, which secrete growth factors: epidermal fibrin fibroblasts and collagen (Hanstan et al., 2008). Berdasarkan fenomena dan teori diatas, peneliti tertarik untuk mengkaji lebih lanjut untuk mengetahui pengaruh ekstrak jarak tintir (*Jatropha multifida* L.) terhadap proses penyembuhan luka pada tikus dengan model perlukaan akut dilihat dari pembentukan fibrin, jumlah fibroblast dan kepadatan kolagen khususnya pada fase inflamasi dan proliferasi.

Materials and Method

Methods This research was carried out with experimental laboratories so that it used a Randomized Post Test Control Group design that used experimental Wistar rats as research subjects. Wistar rats were divided into 3 major groups, namely negative control, positive control, treatment with 10% (*Jatropha multifida* L) extract. Then, each group was divided into 3 groups based on the time dimension, namely the 3rd day, 7th day, and the third day. 14:

- Group K- : Negative control rats with acute injury model treated with ethanol
- Group K+ : Positive control rats with acute wound model treated with 3% Oxitetracycline.
- Group P1 : rats with acute wound models treated with ethanol extract (*Jatropha multifida* L) topically at a concentration of 10%

The period of this research until data collection was carried out for approximately 3 months from March to April 2016. The extraction of the leaves of *Jatropha tintir* was carried out at the Phytochemical Laboratory of the Faculty of Pharmacy Research Activity Center (PKP) Hasanuddin University Makassar. The treatment of rats up to biopsy excision was carried out at the Animal Laboratory, 4th Floor, Faculty of Medicine, Hasanuddin University, Makassar. Paraffin block process, staining using Masson's trichrom method and Van Gieson method and their interpretation of the amount of Fibroblsat, Fibrin formation, and collagen density were carried out at the Anatomical Pathology Section, Hasanuddin University Makassar.

The experimental animals were Wistar rats with the age of 4 weeks to 6 weeks and body weight of 250-300 grams. The Wistar rat is one of the hundred strains originating from the Americas, widely used as experimental animals in research in the fields of medicine, medicine, and veterinary medicine. The wound healing process of mice with an acute wound model was measured by taking a wistar rat wound for anatomical pathology examination to see, the amount of fibroblasts, the formation of fibrin, and the density of collagen from the wound.

The rats were obtained from Laboratory A veterinary UGM Jogjakarta, then after arriving at the animal laboratory of FK Unhas, the groups were divided into 4 large groups and then the cage adaptation was carried out for 3 days. During the experiment, the experimental animals were placed in cages made of wire with a floor area of 30 cm x 50 cm x 15 cm at a temperature of 22 °C (\pm 2 °C) with air humidity (50% – 60%) and given standard feed of 300gr. /head/day and drink in moderation. The standard feed provided was made by the Animal Laboratory, Faculty of Medicine, Hasanuddin University Makassar. The cages were cleaned every day and given lighting in the form of room lights with a 12-hour cycle of turning on and off for 12 hours.

The size of the sample taken according to the WHO is 5 animals and the estimated drop-out is 10% (WHO, 2000), so in this study using a total sample of 6 individuals, each treatment group. The randomization process was: 45 rats were randomly grouped into 3 groups where each had 1 spare rat, consisting of:

Negative Control (K-): 18 mice (5 mice on the 3rd day, 5 mice on the 7th day, 5 mice on the 14th day), Positive Control (K+): 18 mice (5 mice on the 3rd day, 5 mice on the 7th day, 5 mice on the 14th day), Treatment: 18 rats (5 mice on the 3rd day, 5 mice on the 7th day, 5 mice on the 14th day)

Result and Discussions

This research was carried out on May 8, 2016 to July 15, 2016, using 4 laboratories, namely Biopharmaceutical PKP Unhas for the process of making *Jatropha* stem extract (EBJ), Laboratory of Animal Medicine Faculty of Hasanuddin University for the maintenance and treatment of experimental animals, Animal clinic for tissue cutting. and staining and for making slides Histology and research laboratory RSP Unhas assessed fibroblasts, fibrin, and collagen thickness by tissue elisa technique.

In this study, the number of samples was 45 samples consisting of 3 groups, each group consisting of 18 male wistars with 2 tails in each group. The acute modeling was carried out by excision on the left and right back with a diameter of 8 mm using punc biopsy and at each stage of 3 days, 7 days and 14 days sacrifice was performed.

The treatment was carried out on experimental animals in group I (negative control) wistar wounds were treated by applying 0.9% NaCl to the left and right excision wounds, group II (positive control) wistar wounds were treated by applying 10% oxytetracycline to the left and right excision wounds. , Group III Wistar wounds were treated by applying *Jatropha* Stem Extract (EBJ) at a concentration of 10% on the left and right excision wounds. Protokol Penelitian ini telah melalui kaji Penelitian Hewan coba pada Komisi Etik Penelitian Kesehatan (KEPK) Fakultas Kedokteran Hasanuddin University based on Decree Number: 524 /H4.8.4.5.31/PP36-KOMETIK/2016, Furthermore, the research results were analyzed using the SPSS program and described as follows:

Univariate Analysis

Table. 1 Mean value and standard deviation of Fibroblast Count in rats with Acute Injury Model

Observation day	n	Negative Control (Ointment/Vaseline base)	Positive Control (Oxytetracycline Base)	Treatment (10% Distance Stem Extract)	P
3	5	7,6 ± 3,85	3,6 ± 2,07	5,6 ± 3,05	0,081
7	5	6,6 ± 2,88	4,4 ± 1,14	6,8 ± 2,59	0,167
14	5	9,2 ± 4,15	5,8 ± 2,17	9,0 ± 2,92	0,175

Note: p value is obtained from statistical analysis using one way ANOVA

From Table 1, it can be seen that the average number of fibroblasts in rat wounds increased from the 3rd, 7th and 14th days. This can be seen in all treatment groups. Except for the negative control group, where on day 7 there was a slight decrease in mean, fibroblasts compared to day 3. The results of the ANOVA test showed that the increase in the number of fibroblasts on day 14 was not significantly different (0.175) from day 3.

Table. 2 Average Score of Fibrin Formation in Rats with Acute Injury Model

Group	Day 3	Day 7	Day 14
Negative control(ointment base/Vaseline)	1	1	0
	2	2	0
	2	3	0
	2	3	0
	3	3	1
Average	2,0	2,4	0,2
Positive control (base ointment/Oxytetracycline)	1	0	0
	1	0	0
	2	1	1
	2	1	1
	2	1	1
Average	1,6	0,6	0,6
10% jatropa stem extract treatment	1	1	0
	2	1	0
	3	1	1
	3	2	2
	3	3	2
Average	2,4	1,6	1
P Value	0,139	0,024	0,281

Note: P value obtained from statistical analysis using Kruskal Wallis to see the significance.

Looking at table 2, the average score of fibrin formation on day 3 of the rats given the jatropa ointment had a higher fibrin score, compared to the negative control and positive control groups. But this increase was not statistically significant. On the 7th day the treatment group and the positive control were higher in the negative control while on the 14th day the treatment group increased more than the negative control and positive control. It can be concluded that in all treatment groups, except on the 14th day, there was a slight decrease in the fibrin score compared to the 3rd and 7th day.

Table. 3 Collagen density in mice with acute injury models

Group	Seldom		current ly		congest ed		P*	
	N	n						
Day-3								
Negative control	5	5	100	-	-	-	-	0.116
Positif control	5	3	60	2	40	-	-	
10% castor stem extract	5	5	100	-	-	-	-	
Day-7								
Negative control	5	2	40	3	60	-	-	0,089
Positive control	5	-	-	4	80	1	20	
10% castor stem extract	5	-	-	3	60	2	40	
Day-14								
Negative control	5	-	-	4	80	1	20	0,005
Positive control	5	1	20	4	80	-	-	
10% castor stem extract	5	-	-	3	60	2	40	

Note: P value obtained from statistical analysis using Kruskal Wallis to see the significance.

Table 3 shows the density of collagen in mice with an acute treatment model. It was found that on the 3rd day of treatment, most of the rats had not shown the formation of collagen. This was found in the 10%-100% castor extract group, and 100% in the negative control group. While the positive control was 40% which showed moderate collagen formation after the 7th day of treatment, the collagen density was mostly moderate in the negative 60% group, while the positive control was 80% and the jatropa extract group was 10% 60%. The -14 treatments were found to be mostly in the moderate category in the negative 80% group, while the positive control was 80% and the distance rods 10% 60%.

Discussion

The results of this study showed that the average number of fibroblasts in rat wounds increased from the 3rd, 7th and 14th days. This can be seen in all treatment groups. Except for the negative control group, where on day 7 there was a slight decrease in mean, fibroblasts compared to day 3. The results of the ANOVA test showed that the increase in the number of fibroblasts on day 14 was not significantly different (0.175) from day 3.

From the results of the study, the average score of fibrin formation on the third day of rats given Jatropa ointment increased compared to the negative control

and positive control groups but there was no statistically significant difference in this increase. On the seventh day the treatment group and positive control were higher in the negative control while on the fourteenth day the treatment group increased compared to the negative and positive controls. It can be concluded that in all treatment groups, except on the fourteenth day, there was a slight decrease in the fibrin score compared to the third and seventh days.

From the results of this study, the density of collagen in mice with an acute treatment model was obtained. It was found that on the 3rd day of treatment most of the rats had not shown the formation of collagen. This was found in the 10%-100% castor extract group, and 100% in the negative control group. While the positive control was 40% which showed moderate collagen formation after the 7th day of treatment, the collagen density was mostly moderate in the negative 60% group, while the positive control was 80% and the jatropa extract group was 10% 60%. The -14 treatments were found to be mostly in the moderate category in the negative 80% group, while the positive control was 80% and the distance rods 10% 60%.

Conclusions and Recommendations

The 10% jatropa stem extract had no effect on increasing the number of fibroblasts, but significantly decreased fibrin formation on the seventh day and increased collagen density on the fourteenth day. Suggestion Future research should examine the presence of microbes, especially the cause of infection in acute wounds and compare the antimicrobial effect of castor bean with placebo, and further research should look at the occurrence of anti-inflammatory, especially the cause of cell damage in acute wounds and compare the anti-inflammatory effect of jatropa with placebo.

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