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EFFECT OF TOPICAL JATROPHA MULTIFIDA EXTRACT ON TGF-β1 AND EPITHELIEL WISTAR USING ACUTE WOUND MODEL

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ABSTRCT:

Purpose: to determine the effect of giving Jatropha multifida Extract on Tgf-β1 and epitheliel wistar using acute wound model.

Methodology: The method used in this study was the post-test control design group only. The sample consisted of 45 wistar rats divided into 3 groups, namely negative control (Vaseline), positive control (Oxcytetracycline 3%) and Jatropha multifida extract. Data were analyzed by using one way ANOVA test. The research was carried out at the Animal Laboratory of Hasanuddin University, Animal Education Clinic of Hasanuddin University and Hospital of Hasanuddin University.

Main Findings: The results of this study indicated that on the 3rd day, at Tgf-β1 there was a significant difference between the group of negative control - group of Jatropha Extract 10% (p: 0.007), there was a difference between the group of positive control – group of Jatropha Extract 10% (p: 0.004) while on the 7th and 14 th day, there was a difference but it was not significant. Epithelialization on the 3rd day in the negative control group, positive control and Jatropha Extract (p; 0.005), on the 7 th day (p: 0.002) and on the 14 th day (p: 0.002).

Applications: The results of this study could be applied to patients with acute wound model.

Novelty / Originality: The process of healing acute wounds using Jatropha Extract showed better results compared to Oxytetracycline and its control with evidence of an increase in Tgf-β1 levels and a faster re-epitheliel on process in wistar with an acute injury model.

INTRODUCTION:

Wound are broken continuity or damage to tissue integrity as a result of trauma. Wound can be intentionally made for a specific purpose, such as incision wounds in surgery or wound due to trauma (Hantash et al., 2008).

The incidence number of wound is still high, as many as 1.6 million per year are acute wounds due to trauma and wounds from laceration as many as 20 million per year. In America, a study showed that the wound rate was one third of the population. The majority of wounds to the world population are woundsdue to surgery / trauma (48.00%), foot ulcers (28.00%), pressure sores (21.00%). The incidence of wound in Indonesia is still quite high, seen from the increase in traffic accidents in 2015 rose 8% from the previous year resulting in minor wound, serious wound and victims who died.

In general, people still use drugs. The two main groups of antimicrobials that are often used to manage wounds are antiseptic and antibiotic, the use of antiseptic is still controversial because it is toxic to granulation tissue and fibroblasts, and the effectiveness of antiseptic depends on the dilution and also the duration of exposure to the tissue. can deactivate by organic material in wounds such as exudate, blood and pus. Some anti-septic can cause hypersensitivity reactions in some patients, for example in povidone iodine and clorhexidine (Zaenal, et al, 2016).

The process of wound healing is the tissue effort to experience injury to restore normal function and structural integrity after trauma (Leong & Philips, 2012). The wound healing process takes place in all tissues and organs of the body. Healing is a complex process that involves coordinated interaction between various immunological and biological systems that involves several overlapping stages including inflammation, granulation tissue formation, re-epitheliel matrix formation and remodeling (Hantash et al., 2008).

In healing wounds, one of the alternatives used is traditional medicine. The use of traditional medicine in Indonesia is part of the nation's culture and is widely used by the community. At present, although traditional medicine is quite widely used by the community in self-medication (Wijoyo) et al., 2008).

One of the plants used as traditional medicine is Jatropha multifida (Jatropha multifida L.) . There are various uses of the jatropha tree in daily life, including the sap on the tree can be used to treat new wounds and swelling by applying the sap contained in the leaves and stems of the wound (Hariana, 2008). Jatropha multifida contains compounds that can be used as drugs including phenol compounds, flavonoids, saponins and alkaloid compounds (Sundaryono, 2011). Flavonoids function as antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of the bacterial cell membrane.

Jatropha multifida as one of the plants that has been scientifically studied for the evaluation of their wound healing activities in pharmacological models and different patients even benefit in the treatment of infected wounds (Shetty, 2013). Research carried out by Silma et al., (2014), to test antibacterial from Jatropha multifida by using ethyl acetate and methanol extracts found that ethyl acetate extract was more effective as an antibacterial compared to methanol extract. Christina (2014), also conducted a study to look at the differences in the effect of wound care on guinea pigs using tint spacing as compared to using povidon iodine 10%. who use povidon iodine 10% 7-8 days.

Several studies that have been done using Jatropha multifida (Jatropha multifida L.) on wound healing, no one has looked specifically about the epitheliel and Transforming growth factor (Tgf-β1) on the wound healing process, which in general other than looking from a histological perspective The parameters used to assess the wound healing process are molecular parameters namely growth factors including Tgf-β1.

Based on the above phenomena and theories, researchers are interested to study further to determine the effect of the Jatropha multifida on Tgf-β1 and epitheliel in the wound healing process in rat with acute wound model especially in the inflammatory phases and maturation / remodeling.

LITERATURE REVIEW:

Wound healing is a process that involves cellular and biochemical responses both locally and systemically involving a dynamic and complex process of serial coordination including bleeding, coagulation,

initiation of an acute inflammatory response immediately after trauma, regeneration, migration and proliferation of connective tissue and parenchyma cells, and synthesis extracellular matrix proteins, parenchymal remodeling and connective tissue and collagen deposition (T Velnar, 2009). Rat wound with accute wound model is the making of acute wounds inrat through excision wounds measuring 0.8 cm x 0.8 cm on the skin using punch biopsy in the epidermis to the hypodermis / subcutaneous layer.

The cells that play the most role in all these processes are macrophage cells, which function to secrete pro-inflammatory and anti-inflammatory cytokines as well as growth factors, fibroblasts and their ability to synthesize collagen which affects the tensile strength of the wound and replenishes the wound tissue back to its original form, then is followed by skin keratinocyte cells to divide and migrate to form re-epithelialization and cover the woundarea (Faten Khorshid, 2010).

Tgf-β is the level of Tgf-β cytokine polypeptide in tissue serum which is measured using Rat Tgf-β Quunoine Immunoassay ELISA. Epithelialization is a picture of the density of blue fibers epithelialization by painting Massons's trichrom, when observed using an OLYMPUS microscope seri EX51with 100 x magnification in one field of view, the location of the observation of epithelial density is in the excision scar area, then the collagen density is interpreted semi-quantitatively by looking at its density. Painting Masson's tricfrom is a special painting for reticulin fibers (connective tissue fibers that are in the organs).

Syarfati's research in the natural journal (2011) showed that sap of Chinese jatropha was potentially the same as betadin when scab formed in new wounds. Phytochemical studies of various species of Jatropha revealed the presence of alkaloids, cardiac-glycoside, flacanis, flavonoids, tannins, and saponins. (Beatrize C.K. Hirota et al., 2012).

Extract of Jatropha stem referred to in this study is jatropha stem extracted with ethanol solvent. Extracts were made at the Phytochemical Laboratory of the Faculty of Pharmacy Research Activity Center (PKP) of Hasanuddin University in Makassar using maceration method. This method is used because in addition to being more practical, this method can also prevent the loss of important substances during the extraction process, keep the active substance in the stem of the distance, and not damage the active substance, because the heating is below of 500 C.

METHODOLOGY:

This research is an experimental laboratories research with a Randomized Post Test Control Group design that uses wistar rats as research subjects.Wistar rats is diveded into three groups namely group of Jatropha multifida (Jatropha multifida L.), Positive control group (oxytetracikline 3%) dan negative control group (vaselin). This research was conducted for 2 months, from May to July 2016 with research locations in 3 places, Animal Laboratory 4th floor Faculty of Medicine, Hasanuddin University, Laboratory 6th floor Hasanuddin University Hospital and Animal Education Clinic Faculty of Veterinary Medicine, Hasanuddin University . The population in this study were wistar rats. The samples were wistar rats aged 4-6 weeks with a body weight of 250-300 grams. This study has received ethical approval from the Health Research Ethics Committee of the Hasanuddin University Medical School Makassar Number: 524 /H4.8.4.5.31/PP36-KOMETIK/2016

DISCUSSION/ANAYSIS:

Based on the analysis results, in Table 1 it was found that on the 3rd day the negative

control group showed an average (mean) Tgf-β1 (83.82 ± 4.17) and an increase on the 7th day and continued to increase until the 14th day with an average (100.92 ± 18.80). In the positive control group given theraphy of oxcytetracycline 3%, the 3rd day3 showed an average of Tgf-β1 (81.80 ± 11.61) this number continued to increase until the 7th day and slightly decreased on the 14 th day observation with a difference (1.24). In contrast to the two previous groups, in the third group who were given Jatropha Extract therapy the amount of Tgf-β1 tended to increase on the 3rd day observation with an average (105.43 ± 13.16) this was the highest number on the day 3rd among the three treatment groups, the average Tgf-β1 in this group continued to decrease on the 7th day (97.66 ± 8.90) until the 14 th day (87.09 ± 13.29).

At each time of observation there were differences between treatment groups, to see the level of significance of each group's significance each time the observation was carried out statistical tests and obtained on the 3rd day the value ($p < 0.05$) Whereas on the 7 th day and and day 14 th there was a change but statistically there was no significant difference ($p > 0.05$).

Table 1. Overview of Tgf-β1 levels in Wistar Rats with an Acute Wound Model

Group	Mean	Std.Deviation
The 3 day		
Negative Control	83,82	4,17
Positive Control	81,80	11,61
Jatropha multifida extract 10%	105,43	13,16
The 7 day		
Negative Control	93,92	24,19
Positive Control	106,51	6,53
Jatropha multifida extract 10%	97,66	8,90
The 14 day		
Negative Control	100,92	18,80
Positive Control	105,27	5,58
Jatropha multifida extract 10%	87,09	13,29

Source: Research Result,2016

Table 2 showed that epitheliel in the negative group and positive group on the 3rd day looks the same, ie re-epitheliel has not formed yet (60%), re-epitheliel <50% (40%) is different from the third group which is given Jatropha multifida extract on the 3rd day of the results observations began to show re-epitaliel ≥ 50 in all samples (100%).

On the 7th day to the 14th day in each group the epitheliel picture showed an increase, in the negative control group all samples experienced re-epitheliel <50 (100%) and on the 14 th day re-epitheliel ≥50% had reached (80%) . In the group given Oxcytetracycline 3% on the 7 th day which showed re-epitheliel ≥50 reached (60%) and on the 14th day the epitheliel picture in all five samples (100%) had shown re-epitheliel ≥50%. Whereas in the group given the Jatropha multifida Extract ointment on the 7 th day as much as 80% had showed the closure of the excision wound and the formation of

keratinization began to be seen on the 14 th day by 60%. The Jatropha multifida extract group showed better epitheliel until day 14 th compared to the negative and positive control groups.

Tabel 2. The description of epitheliel on Wistar rat with an acute wound model

Group	No reepiteliz ation		Re-epitheleli < 50%		Re-epitheliel ≥ 50%		Closing of excicion		Keratinizati on		n	p
	n	%	n	%	n	%	n	%	n	%		
The 3 Day												
Negative Control	3	60	2	40	-	-	-	-	-	-	5	
Positive Control	3	60	2	40	-	-	-	-	-	-	5	0,005
Jatropha Extract 10%	-	-	-	-	5	100	-	-	-	-	5	
The 7 Day												
Negative Control	-	-	5	100	-	-	-	-	-	-	5	
Positive Control	-	-	2	40	3	60	-	-	-	-	5	0,002
Jatropha Extract 10%	-	-	-	-	-	-	4	80	1	20	5	
The 14 Day												
Negative Control	-	-	1	20	4	80	-	-	-	-	5	
Positive Control	-	-	-	-	5	100	-	-	-	-	5	0,002
Jatropha Extract 10%	-	-	-	-	-	-	2	40	3	60	5	

Source : Research Result,2016

This study showed that there was an increase in Tgf-β1 levels and a good re-epitheliel process in wistar rat wounds with an acute wound model with the administration of Jatropha multifida stem extract therapy.

The group given Jatropha multifida extract (group III) had a significant increase in Tgf-β1 levels on the 3rd day. (Barrientos, et al, 2008) stated that Tgf-β1 originated from platelets, keratonocytes, macrophages, lymphocytes, increased fibroblasts in acute wounds that function Inflammation, granulation tissue formation, matrix formation and remodeling. Tgf-β1 is a pro-inflammatory mediator that stimulates neutrophils as inflammatory cells, fibroblasts and epithelial cells. The high pro-inflammatory mediator of the wound, indicates that the inflammatory process is in progress, if the level of pro-inflammatory mediator's decreases, indicating the wound is starting to improve. Decreased mediator pro-inflammatory in the blood indicates the wound is starting to improve (Zaenal, et al. 2011). Physiologically, when there is a wound in the inflammatory phase the body has produced one of the growth factors, Tgf-β1 which will contribute to tissue proliferation. The anti-inflammatory activity of flavonoids shortens the occurrence of inflammatory reactions due to inhibition of

cyclooxygenation and lipoxygenase which causes the number of inflammatory cells to migrate to the wound tissue to be limited and was not inhibit the ability of TGF-β to proliferate, thereby making the TGF-β proliferation process (Igbinosa, O. O 2009). Provision of Jatropha multifida extract will help increase Tgf-β1 levels in the inflammatory process. Thereby, Jatropha multifida extracts indirectly accelerate wound healing with its antibacterial effect with the compound content, namely phenol, flafonoid, saponin and alkaloid (Sundaryono, 2011).

Epitheliel in the negative control group l and positive control group tended to be the same, differences in epitheliel formation began to occur on the 7th day and the 14 th day, whereas in the Jatropha multifida Extract group on the 3rd day re-epitalized ≥ 50%, and continued to experience wound closure and the formation of keratin and hair follicles until the 14 th day which indicates that the wound healing process in wistar rats is going well.

The addition of epithelial thickness in the group treated with 10% spacing castor extract indicates there was a synergistic relationship between the content of the spacing castor compound with biochemical mediators involved in the wound healing process.

Re-epitheliel occured a few hours after injury. The wound epithelium consisted of basal cells detaches from the base and moves to fill the wound surface. The place is then filled by new cells formed from the process of mitosis. The migration process only occured towards a lower or flat direction. This process only stops after the epithelium touches each other and closes the entire wound surface. With the wound surface closed, the process of fibroplasia by forming granulation tissue will also stop and the maturation process will begin in the interim phase. The process of complete reepithelialization is less than 48 hours on a cut wound whose edges are close together and require a longer time for wounds with wide defects (Victor, 2010).

This study is in line with research conducted by Jayadi (2015), on wound healing of diabetic wistar rats induced by curcumin, showing that curcumin has an antibacterial effect by increasing reepithelization and Tgf-β1 levels.

CONCLUSION:

Based on the results of the analysis it was concluded that: an increase in Tgf-β1 levels and a faster re-epitheliel process in wistar rats with an acute wound model compared with a negative control group (vaseline) and a positive control group (Oxytetracycline 3%). Further research is needed to extract the Jatropha multifida in various concentrations for more effective wound healing.

LIMITATION AND STUDY FORWARD:

The molecular parameters of wound healing used in this study were limited, namely Transforming growth factor (Tgf-β1), so it was necessary to add other wound healing molecular parameters for comparison such as pro-inflammatory citocaine such as tumor necrotic factor (TNF-α) and Interleukin-1.Preparations extracts that are limited to use only preparations in the form of Topical cream need to be tested in the future to tint the Jatropha extract in the form of hydro gel and ointment with different extract presets.

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