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Effects of Methanolic *Jatropha multifida* L. Extract in Wound Healing Assessed by the Total Number of PMN Leukocytes and Fibroblasts

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Abstract

The aim of this study was to evaluate the effects of methanol extract of *Jatropha multifida* leaves on the wound healing process and to investigate the wound healing activity based on reduced numbers of PMN (polymorpho nuclear) leukocytes and increased numbers of fibroblasts. Methanol extract of dried leaves of *J. multifida* was used in the wound healing activity studies. The study subjects were 36 white male Sprague Dawlay rats aged 2 months with 150-200 gram body weight. The subjects were divided into 4 groups and experimentally injured: Group I (negative control) underwent injury without subsequent treatment; group II (positive control) received topical treatment with Bethasone-N after injury; group III (solvent control) was treated with 70% methanol; group IV (treatment group) was treated with 10 mg methanol extract of *J. multifida* Each group consisted of 3 rats, which were decapitated on days 3, 6, and 13 after the start of treatment. Histological preparation was stained with hematoxyline-eosin (HE) and was continuously examined by counting the numbers of PMN leukocytes and fibroblasts as indicators of wound healing on days 3, 6, and 13 of treatment. The study showed lower numbers of PMN leukocytes in subjects treated with the extract of *J. multifida* as compared to the other groups. The numbers of fibroblasts were significantly higher on days 6 and 13 of treatment. The treatment of injuries with methanol extract of leaves from *J. multifida* provided better results compared to the other groups in our study.

Abstrak

Pengaruh Ekstrak Metanol Daun *Jatropha multifida* L. pada Proses Penyembuhan Luka Sayat Melalui Penilaian Jumlah Leukaosit PMN dan Fibroblas. Tujuan penelitian ini adalah untuk menilai proses penyembuhan luka dengan menggunakan ekstrak metanol daun *Jatropha multifida* L. berdasarkan mekanisme penurunan jumlah leukosit PMN dan peningkatan jumlah sel fibroblas. Bahan yang digunakan dalam penelitian ini adalah ekstrak metanol dari daun *J. multifida* Subyek penelitian terdiri dari 36 ekor tikus putih jantan galur *Spraque Dawlay* umur 2 bulan dengan berat badan sekitar 150-200 g. Hewan coba dibagi menjadi 4 kelompok. Kelompok I (negatif kontrol) merupakan kelompok hewan coba yang dilukai tanpa diobati; kelompok II (kontrol positif) merupakan kelompok hewan coba yang diobati dengan Bethasone-N; Kelompok III (kontrol pelarut) merupakan kelompok yang diobati dengan alkohol 70% sedangkan kelompok IV (kelompok perlakuan) merupakan kelompok yang diobati dengan meneteskan 10 mg ekstrak metanol daun *J. multifida*. Setiap kelompok terdiri dari 3 ekor tikus yang masing-masing dibagi lagi menjadi kelompok waktu dekapitasi pada hari ke 3, 6, dan 13. Pada jaringan luka dibuat sediaan histologi dengan pewarnaan HE dan dilanjutkan dengan menghitung jumlah leukosit PMN dan fibroblas. Pada penelitian ini memperlihatkan bahwa penurunan jumlah leukosit PMN pada kelompok perlakuan dengan ekstrak metanol daun *J. multifida* relatif lebih baik dibandingkan dengan kontrol negatif, kontrol positif dan kontrol pelarut. Peningkatan jumlah fibroblas terjadi pada hari ke 6 dan 13 setelah perlakuan. Ekstrak metanol daun *J. multifida* dapat mengobati luka sayat lebih baik dibandingkan dengan kontrol negatif, kontrol positif dan kontrol pelarut.

Keywords: fibroblast, Jatropha multifida, PMN leukocytes, wound healing

1. Introduction

Wound healing is one of the most important post-operative processes and various research activities have been undertaken to find the best way to improve and accelerate wound healing [1]. About six million people in the world are suffering from chronic wounds [2]. The government of the United States of America bears the costs of wound treatment of more than one billion dollars per year, and at the global level the cost is seven billion dollars for long-term treatment of wounds [2-3]. Comparable data for Indonesia are as yet not available.

Since economic and social costs of existing wound treatment are high for patients and goverments, it is necessary to find new medicines, which can be applied as alternative or complementary therapies.

Chemical substances such as synthetic steroids are usually applied in wound treatment. However, the side effects, such as decreasing the production of TGF-b and IGF-I, occur. In addition, steroids have an impact in decreasing desposition of collagen [4]. The price and availability of chemical medicines should also be taken into account in developing countries like Indonesia, especially when dealing with large populations in remote areas. Therefore, it is essential to find sources for alternative medicines.

In recent years, it has been quite popular among rural population to use herbs and their products as medicine to treat wounds. The reason is that herbs contain many beneficial pharmacological active compunds and it is widely accepted that such compounds are more tolerable and cheaper than synthetic chemicals and that they can be processed into safe drugs [5]. One of the traditional plants widely used for wound treatment in America, Asia and Africa is *Jatropha multifida* [6-11], although there are not many research results that can support the effectiveness and safety of this plant as a traditional medicine to treat wounds. Studies on other *Jatropha* species have already been published, such as *J. curcas* for wound healing [12-13] and as a medicine in dentistry [14], *J. maheshwarii* as a medicine in dermatology and dentistry [15], *J. glandulifera* for wound treatment with a wide range of therapeutic effects, such as antiinflammatory, antimicrobial activity, antioxidant, antithrombosis and antitumor [16].

The aim of the present study was to prove scientifically the effectiveness of *J. multifida* leaf extract in different phases of the wound healing process based on the counting of the total numbers of PMN leukocytes and fibroblasts.

2. Methods

Plant material. Leaves of *J. multifida* were collected in Pariaman, West Sumatra. The fresh leaves were chopped into small pieces, sun-dried and crushed in a blender. An amount of 1769 g of powdered leaves was macerated with 70% methanol for 24 hours and stirred intermittently. Evaporation of the resulting solution *in vacuo* yielded 19.28 g of a viscous dark green methanol extract (yield 10.9 %).

Wound creation. The Spraque Dawley rats were obtained from the *Laboratorium Non Ruminansia dan Satwa Harapan, Fakultas Peternakan, Institut Pertanian Bogor*. Thirty six male rats with the same age of two months and 200-250 gram body weight were divided into four groups. Group I was used as the negative control group (without any treatment), group II (positive control) was treated with Bethasone-N, group III (solvent control) was treated with 70% methanol as the solvent of the extract, and group IV was treated with 10 mg methanol extract of *J. multifida* leaves dissolved in 70 % methanol. The applied doses were obtained from preliminary studies with varying doses of 10 mg, 20 mg, and 40 mg.

Each group consisted of 3 rats. Prior to cutting, the rats were anesthetized using ketamine and the back side hair was shaved about 3 x 3 cm wide and marked for the incision. The skin was lifted using two pairs of tweezers in order to prevent further extension of the trauma. Cuts were made along the marked line about 1 cm long and 1-2 mm deep into the skin and hypodermis using a scalpel. The rats were decapitated after a period of 3, 6, and 13 days and the process of wound healing was assessed. Day 3 was defined as the inflammatory phase, day 6 as the proliferative phase, and day 13 as the remodeling phase.

After cuts were made, each rat was treated according to the group. The histological preparations of the scar tissue were prepared and mounted on slides, which were stained with hematoxylin-eosin (HE). The total numbers of PMN leukocytes and fibroblasts were counted from each slide.

3. Results and Discussion

The number of PMN leukocytes. The mean values of the numbers of PMN leukocytes for the four treatment groups are presented in Table 1. On day 3 (inflammatory phase), the group treated with the leaf extract of *J. multifida* showed the lowest number of PMN leukocytes, followed by the solvent control, positive control, and negative control. On day 6 (proliferative phase), the number of PMN leukocytes decreased in the group treated with the extract and in the negative control group, while the positive and solvent control groups showed the opposite effect. On day 13 (remodeling phase), PMN leukocytes decreased in all groups in contrast to days 3 and 6. Again, the group treated with the leaf extract of *J. multifida* showed the

lowest number, followed by the solvent control group, positive control and negative control.

In the inflammatory phase, which is represented by day 3 in this study, pro-inflammatory PMN leukocytes migrated to the wound area by chemotaxis. This process was characterized by the infiltration of neutrophils, macrophages and lymphocytes. The main function of PMN leukocytes in this stage was to minimize the bacterial contamination of the wound. Macrophages played a dual role in wound healing.

In the proliferative and remodeling phases, PMN leukocyte numbers decreased with reduction of bacterial contamination in the injured area [17-19]. This is consistent with the results in the group treated with the leaf extract of *J. multifida* and the negative control. In the solvent control and positive control groups the inflammatory phase was obviously prolonged. At day 13, the inflammatory processes were reduced in all goups as indicated by generally decreased numbers of PMN leukocytes (Table 1).

The number of fibroblasts. The mean values of fibroblast numbers of all groups are shown in Table 2. The group treated with the leaf extract of *J. multifida* showed the highest fibroblast numbers in all phases of wound healing, followed by the negative control, then the positive control and finally the solvent control groups.

In the proliferative phase in this study, which is represented by day 6 of wound healing, the number of fibroblasts increased and at this stage matrix proteins, fibronectin, hyaluronan, collagen and proteoglycans were produced. These components helped rebuilding the extracellular matrix, which supported consecutive cell growth. This process was important in tissue repairing. In the remodeling phase, which is represented in our study by day 13, the number of fibroblasts decreased in all treatment and control groups (Table 2) [19].

In the inflammatory phase, the numbers of PMN leukocytes were lower and in the proliferative phase, the numbers of fibroblasts were higher in the group treated with the leaf extract of *J. multifida* than the values of all other groups. It means that the inflammatory phase was shorter in this group and the proliferation started earlier and was more intensive. This reaction was attributed to the content of secondary metabolites found in the methanol extract of leaves of *J. multifida*.

Nwokocha *et al*. [7] suggest that the leaves, roots, seeds and stems of *J. multifida* contain secondary metabolites, such as alkaloids, tannins, flavonoids, saponins and phenols. From the quantitative test, leaves of this plant are known to have the highest contents of secondary metabolites with various activities, such as: saponins serve as antibiotics and antioxidants [20], tannins have a stringent characteristic, which can cause precipitation of proteins on the cell surface, so that the permeability of the cell membrane decreases without disrupting the integrity of membrane function and furthermore, tannins act as antiseptics [21].

Table 1. Mean Values of the Numbers of PMN Leukocytes for 10 High-Power Fields (HPF)

Day	N	The number of PMN leukocyte cells for 10 high-power fields (mean ± standard deviation)			
		Negative control group	Positive control group	Solvent control group	Treatment group with leaf extract of *J. multifida* L
3	3	110.00±4.558	92.00±3.61	71.00±7.21	45.57±4.00
6	3	63.67±1.528	109.67±5.51	78.00±5.56	31.00±2.00
13	3	60.00±6.245	56.33±2.52	52.33±2.31	15.67±1.53

Table 2. Mean Values of the Numbers of Fibroblasts for High-Power Fields (HPF)

Day	N	The number of fibroblasts for high-power fields (mean ± standard deviation)			
		Negative control group	Positive control group	Solvent control group	Treatment group with leaf extract of *J. multifida* L
3	3	33.00±4.58	31.67±2.01	28.00±3.46	67.67±2.52
6	3	99.67±4.51	91.00±7.00	68.33±21.36	112.33±10.26
13	3	81.67±9.45	73.33±4.16	59.00±2.65	87.00±23.39

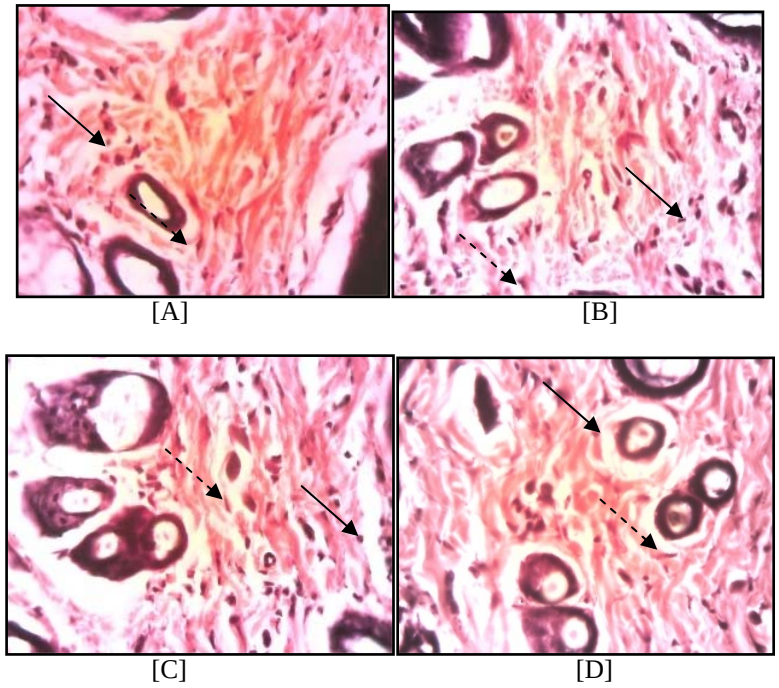

Figure 1. Microscopic Image of PMN Leukocytes and Fibroblasts in the in the Group Treated with the Leaf Extract of *J. multifida* L. (A), the Solvent Control Group (B), Positive Control Group (C), and Negative Control Group (D) on Day 6 of the Wound Healing Process. Arrows Indicate PMN Leukocytes and Dashed Arrows Indicate Fibroblasts

Several studies have reported on the efficacy of the compounds and the content of these plant parts including resin containing cyclic peptide compounds, and phenolic glycosides [22], labaditin [23], multifidol [24], multifidol glucosidase [25], diterpenoid, multidion [22], and proantrocianidin multifidin. These compounds found in the saps are believed to possess anti-complement activity [25].

The trunk of *J. multifida* has been reported to contain multifidanol that can inhibit the growth of *B. subtilis* and *E. coli*; multifidenol can inhibit the growth of *Streptococcus aureus.* Either compound has cytotoxic and antibacterial activities [26]. Moreover, the trunk contains the diterpenoid multifolone and (4E)-jatrogrossidentadion acetate [27].

4. Conclusions

The healing process of cuts using methanol extract of *J. multifida* leaves shows promising results, it was better compared to those shown by the application of Bethasone-N and the controls. The numbers of pro-inflammatory PMN leukocytes were lower, the inflammatory phase was shorter and the numbers of proliferative fibroblasts were higher.

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