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THE EFFECTIVENESS OF ETHANOL EXTRACT OF BINAHONG LEAVES ON DIABETIC WOUND HEALING

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ABSTRACT

This research aimed to study the effectiveness of ethanol extract of binahong leaves on blood glucose, insulin, blood chemical profiles (serum glutamic pyruvate transaminase=SGPT, serum glutamic oxaloacetic transaminase=SGOT, ureum, and creatinine), and skin histopathology in diabetic rat. A total of 20 male Wistar rats aged 3 months (± 250 gram) were divided into five groups, with four rats in each group. Group I (non-diabetic control) was injected with 0.1 M sodium citrate buffer, while group II (diabetic control), III, IV, and V were injected with single dose of Streptozotocin (STZ) at dose 40 mg/kg intraperitoneally (IP). One week after the injection, the dorsal skin of the rats were excised. Group I and II were given cream topically and 1% sodium carboxymethyl cellulose (NaCMC) orally, group III was given 50% ethanol extract of binahong leaves (EEB) topically and 1% NaCMC orally, group IV was given cream topically and EEB 300 mg/kg orally, and group V was given 50% EEB topically and EEB 300 mg/kg orally. These treatments were continued for 14 days. Blood samples were obtained at the end of study to examine blood glucose, insulin, and blood chemical profiles (SGOT, SGPT, ureum, and creatinine). Examination of skin histopathology and leukocyte count were also done. The result showed that blood glucose, insulin, SGOT, SGPT, and ureum level of diabetic rats given topical or oral EEB did not significantly different from diabetic control group, even though blood glucose, insulin, SGOT, SGPT, and ureum level of diabetic rats given topical and oral EEB were found lower compared to diabetic control group. Administration of EEB 300 mg/kg orally in diabetic rats could lower creatinine level significantly ($P<0.05$). Histopathological examination of dorsal skin of diabetic rats which were given EEB topically showed the decrease of fibroblast proliferation, leukocyte infiltration, and hemorrhage in dermis area. Leukocyte count on skin tissue was significantly lower ($P<0.05$) in diabetic rats given EEB. In conclusion, topical or oral administration of EEB can help healing process in diabetic wound.

Key words: binahong leaves, diabetes, ethanol extract, wound

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui kadar glukosa, insulin, kimia darah (serum glutamic pyruvate transaminase=SGPT, serum glutamic oxaloacetic transaminase=SGOT, ureum, kreatinin) dan histopatologi kulit pada tikus diabetes setelah diberi ekstrak etanol daun binahong. Dalam penelitian ini digunakan 20 ekor tikus wistar jantan berumur tiga bulan (± 250 g) yang dibagi secara acak menjadi lima kelompok, setiap kelompok terdiri atas empat ekor. Kelompok I (kontrol non diabetes) diinjeksi 0.1 M sodium citrate buffer, sedangkan tikus kelompok II (kontrol diabetes), III, IV, dan V diinjeksi tunggal dengan streptozotocin (STZ) 40 mg/kg secara intraperitoneal (IP). Satu minggu setelah injeksi, kulit bagian punggung seluruh tikus dilukai secara eksisi. Kelompok I dan II diberi krim topikal dan sodium carboxymethyl cellulose (NaCMC) 1% per oral, kelompok III diberi ekstrak etanol daun binahong (EEB) 50% secara topikal dan NaCMC 1% per oral, kelompok IV diberi krim topikal dan EEB 300 mg/kg per oral, dan kelompok V diberi EEB 50% secara topikal dan EEB 300 mg/kg per oral. Perlakuan dilaksanakan selama 14 hari. Pada akhir perlakuan, darah diambil untuk pemeriksaan kadar glukosa, insulin, dan kimia darah, pemeriksaan histopatologis kulit, dan penghitungan jumlah leukosit. Hasil penelitian menunjukkan kadar glukosa, insulin, SGOT, SGPT, dan ureum tikus diabetes yang diberi EEB secara topikal maupun oral tidak berbeda signifikan dengan tikus kontrol diabetes, meskipun demikian, kadar glukosa, insulin, SGOT, SGPT, dan ureum tikus diabetes yang diberi EEB cenderung lebih rendah dibandingkan tikus kontrol diabetes. Pemberian EEB 300 mg/kg secara per oral pada tikus diabetes dapat menurunkan kreatinin secara signifikan ($P<0,05$). Gambaran histopatologi kulit tikus diabetes yang diberi EEB menunjukkan adanya proliferasi fibroblas, infiltrasi leukosit, dan menurunnya hemoragi di daerah dermis. Jumlah leukosit pada jaringan kulit secara signifikan lebih rendah ($P<0,05$) pada tikus diabetes yang diberi EEB. Dapat disimpulkan bahwa pemberian EEB secara topikal maupun oral dapat memperbaiki luka diabetes.

Kata kunci: daun binahong, diabetes, ekstrak etanol, luka

INTRODUCTION

Diabetes is a metabolic disease characterized by chronic hyperglycemia that can lead to pathophysiological disorders and abnormalities of carbohydrate, protein, and fat metabolism (Ozougwu *et al*., 2013). Prevalence of diabetes worldwide has increased for the last three decades and diabetes is growing rapidly in developing countries (WHO, 2016). Diabetes can also occur in pets, especially in dogs and cats. The prevalence of diabetes in animals continues to increase due to an increased prevalence of obesity. Most cases of diabetes mellitus in cats have similarities with type 2 diabetes mellitus in humans due to β-cell dysfunction and insulin resistance. The β-cell dysfunction can be caused by amyloid islet deposition, glucose toxicity and damage caused by reactive oxygen species (ROS). Factors that play role in insulin resistance include obesity (Sparkes *et al*., 2015). According to Rucinsky *et al*. (2010), diabetes mellitus in dogs is also caused by pancreatic β-cell dysfunction. The development of β-cell can be impaired due to immune-mediated destruction, vacuolar degeneration, and pancreatitis. In female dogs, diabetes may occur temporarily due to the effects of insulin resistance in the diestrous phase.

Wounds often occur in diabetics and require longer healing time compared to non-diabetic patients (Waugh and Sherratt, 2006). The process of wound healing under diabetic conditions may be impaired due to dysfunction of inflammatory responses, decreased granulation tissue formation, disturbed angiogenesis,

and increased apoptosis of fibroblasts. Diabetic wound healing is characterized by numerous polymorphonuclear cells which are characteristic of persistent inflammation and decreased connective tissue formation (Desta *et al*., 2010).

According to Lerman *et al*. (2003), decreased production of growth factors (keratinocyte growth factor/KGF, vascular endothelial growth factor/VEGF, platelet-derived growth factor/ PDGF), and an increase in microbial count may interfere wound healing process in diabetic. In addition, diabetics often have peripheral vascular disease and polyneuropathy that can slow wound healing. Several studies also mentioned that diabetic wound has proangiogenic growth factor deficiency, prolonged inflammatory phase, cell migration disorder, and wound contraction disorder. Permanent cell damage can be attributed to accumulation of advanced glycosylation end-products (AGEs) in cells exposed to chronic hyperglycemia and oxidative damage due to overproduction of mitochondrial oxidative stressors. The abnormal activity of VEGF and the response to hypoxia greatly affects the healing process of diabetic wounds. In the absence of sufficient angiogenic response, cell proliferation and matrix deposition may be delayed due to deficiency of new blood vessels.

Management of diabetes requires special attention and expensive cost. Therefore, various alternative treatments are currently developed, for example, the use of traditional medicine such as binahong leaves. Binahong (*Anredera cordifolia* Ten.) Steenis) is a plant that is often found in the yard of the house and has antibacterial, anti-obesity, antiviral, antidiabetic, antiulcer, and anti-inflammatory properties (Miladiyah and Prabowo, 2012). Administration of methanol extract of binahong leaves dose 200 mg/kg for 14 days in rats could lower blood sugar level (Sukandar *et al*. 2011) and healed the wound (Manoi, 2009). Miladiyah and Prabowo (2012) reported that the healing of the wound which were given topical application of ethanol extract of binahong leaves 20% and 40% were better than control rats which were given povidone iodine. Therefore, research on utilization of binahong leaves to heal diabetic wounds is very necessary as an attempt to find other alternative therapies that are cheap and safe.

MATERIALS AND METHODS

This study used 20 male Wistar rats aged three months (± 250 grams) divided randomly into five groups (I, II, III, IV, and V), each group consisting of four rats. Rats in group I (non-diabetic control) were injected with 0.1 M sodium citrate buffer, whereas rats in group II (diabetic control), III, IV, and V were injected with intraperitoneal (IP) streptozotocin (STZ) at dose of 40 mg / kg. Before the injection, all of the rats were fasted for 12 hours. One week after the injection, the rats were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg). Subcutaneous wound excision were performed using biopsy punch (5 mm diameter) on the backs of all rats. Group I and II were treated with cream topically and 1% sodium carboxymethyl cellulose (NaCMC) orally while group III was given 50% ethanol extract of binahong (EEB) topically and 1% NaCMC orally, group IV was given cream topically and EEB at dose of 300 mg/kg orally, group V was given 50% EEB topically and 300 mg/kg EEB orally. Treatment was carried out for 14 days. At the end of treatments, rats were anesthetized with ketamine (50 mg/kg) and xylazin (5 mg/kg) intramuscularly, then blood was collected from retroorbital sinuses for glucose, insulin, and blood chemistry (serum glutamic pyruvate transaminase =SGPT, serum glutamic oxaloacetic transaminase =SGOT, ureum, creatinine) tests. The excised skin tissue was removed and fixed in 10% formalin for histopathological examination (using hematoxylin eosin staining). The number of leukocytes in excised skin tissue is calculated on six different fields of view (400x magnification). Blood test results and the number of leukocytes in skin tissue were statistically analyzed using one way analysis of variance (ANOVA), while histopathological examination was analyzed descriptively. All activities in this study had been approved by the Ethical Clearance Commission, Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University (No.460/KEC-LPPT/IV/2016).

RESULTS AND DISCUSSION

Diabetes is characterized by hyperglycemia due to impaired insulin secretion, insulin action or both (Ozougwu *et al*., 2013). It may lead to other complications such as hyperlipidemia, hypertension, atherosclerosis, retinopathy, neuropathy and nephropathy (Al-Qudah *et al*., 2016). Wound is commonly occurred in diabetics and requires longer recovery time compared to non-diabetic patients (Waugh and Sherratt, 2006).

Glucose and Insulin Levels

The results showed that blood glucose in diabetic control rats was 355.55±74.14 mg/dL. It was significantly higher ($P<0.05$) than non-diabetic control group blood glucose (101.9±8.23 mg/dL). Blood glucose levels of diabetic control rats (355.55±74.14 mg/dL) and diabetic rats given topical EEB (249.13±120.37 mg/dL), oral EEB (257.13±139.40 mg/dL), or combination (356.88±78.52 mg/dL) did not show any significant difference ($P>0.05$). However, diabetic rats given topical and oral EEB had lower glucose level than diabetic control rats. Insulin levels throughout the treatment did not show any significant difference ($P>0.05$) (Table 1).

Streptozotocin (STZ) is a substance which has diabetogenic, hepatotoxic, and nephrotoxic properties and could cause ulceration of the stomach. The diabetogenic nature of STZ is a direct result of irreversible damage to pancreatic β-cell, β-cell dysfunction, and decreased mass of β-cell resulting in degranulation and loss of the ability to secrete insulin (Zafar and Naqvi, 2010; Qinna and Badwan, 2015). Zafar and Naqvi (2010) explained that STZ causes

cellular necrosis and pancreatic β-cell destruction through direct alkylation mechanism and led to hyperglycemia at doses of 45 mg/kg. The same was explained by Mohan *et al*. (2013) that the cause of pancreatic β-cell death was DNA alkylation which results in decreased synthesis and release of insulin. The fragmentation of DNA is due to the production of ROS. Streptozotocin selectively destroys pancreatic β-cell that secrete insulin, so that pancreatic cells become less active and can lead to diabetes mellitus.

Study conducted by Sukandar *et al*. (2011) showed that administration of methanol extract of binahong leaves with dose of 200 mg/kg for 14 days in diabetic rats can lower blood glucose level. Makalalag *et al*. (2013) also explained that binahong leaves extract with dose of 1.8 g/kg body weight can reduce blood glucose levels induced by sucrose. In this study, blood glucose levels of diabetic rats which were given combinations of EEB topically and orally tend to be higher than those were only given EEB topically or orally alone. According to Sukandar *et al*. (2011), the test substance in the form of an extract might contain a mixture of active compound and its antagonists; therefore, in higher doses there might be decreased antidiabetic effect due to increased antagonistic effect.

Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetic Transaminase (SGOT)

SGOT and SGPT levels of diabetic control rats (349.58±110.70 U/L and 223.00±89.04 U/L) were not significantly different (P>0.05) compared to non-diabetic control rats (123.93±11.29 U/L and 62.35±25.62 U/L). However, SGOT and SGPT levels tend to be higher in diabetic control rats. SGOT and SGPT levels of diabetic control rats (349.58± 10.70

Table 1. Average of blood glucose and insulin level in non-diabetic and diabetic rats after administration of topical 50% EEB and/or oral EEB 300 mg/kg for 14 days of intervention

Groups	Glucose (mg/dL)	Insulin (ng/mL)
I	101.90±8.23[a]	225.98±108.77
II	355.55±74.14[b]	192.93±36.23
III	249.13±120.37[b]	134.89±37.67
IV	257.13±139.40[b]	187.04±16.06
V	356.88±78.52[b]	165.12±25.48

[a, b]Different superscripts within the same column indicates significant differences (P<0.05). I= Sodium citrate buffer injection + topical cream + oral NaCMC, II= STZ injection + topical cream + oral NaCMC, III= STZ injection + topical 50% EEB + oral NaCMC, IV= STZ injection + topical cream + oral EEB 300 mg/kg, V= STZ injection + topical 50% EEB + oral EEB 300 mg/kg

Table 2. Average means of SGOT and SGPT blood level in non-diabetic and diabetic mice after administration of topical EEB 50% and/or oral EEB 300 mg/kg for 14 days of intervention

Groups	SGOT (U/L)	SGPT (U/L)
I	123.93±11.29[a]	62.35±25.62[a]
II	349.58±110.70[ab]	223.00±89.04[ab]
III	174.83±49.00[ab]	116.13±38.17[a]
IV	380.65±259.60[ab]	195.23±98.50[ab]
V	478.35±335.25[b]	360.85±239.55[b]

[a, ab, b]Different superscripts within the same column indicates significant differences (P<0.05). I= Sodium citrate buffer injection + topical cream + oral NaCMC, II= STZ injection + topical cream + oral NaCMC, III= STZ injection + topical 50% EEB + oral NaCMC, IV= STZ injection + topical cream + oral EEB 300 mg/kg, V= STZ injection + topical 50% EEB + oral EEB 300 mg/kg

Table 3. Average of ureum and creatinine level in non-diabetic and diabetic rats after administration of topical 50% EEB and/or oral EEB 300 mg/kg for 14 days of intervention

Groups	Ureum (mg/dL)	Creatinine (mg/dL)
I	31.95±4.83[a]	0.24±0.02[ab]
II	107.23±45.32[b]	0.31±0.04[a]
III	95.65±48.25[b]	0.28±0.08[a]
IV	100.80±19.06[b]	0.18±0.04[b]
V	115.15±27.88[b]	0.27±0.05[a]

[a, ab, b]Different superscripts within the same column indicates significant differences (P<0.05). I= Sodium citrate buffer injection + topical cream + oral NaCMC, II= STZ injection + topical cream + oral NaCMC, III= STZ injection + topical 50% EEB + oral NaCMC, IV= STZ injection + topical cream + oral EEB 300 mg/kg, V= STZ injection + topical 50% EEB + oral EEB 300 mg/kg

Table 4. Average of leukocyte count in wounded skin tissue in six different plane fields of non-diabetic and diabetic rats after administration of topical 50% EEB and/or oral EEB 300 mg/kg for 14 days of intervention

Groups	Leukocyte count
I	16.71±5.82[a]
II	36.88±17.30[b]
III	15.67±2.34[a]
IV	23.17±4.67[a]
V	20.84±3.83[a]

[a, b]Different superscripts within the same column indicates significant differences (P<0.05). I= Sodium citrate buffer injection + topical cream + oral NaCMC, II= STZ injection + topical cream + oral NaCMC, III= STZ injection + topical EEB 50% + oral NaCMC, IV= STZ injection + topical cream + oral EEB 300 mg/kg, V= STZ injection + topical 50% EEB + oral EEB 300 mg/kg

U/L and 223.00±89.04 U/L) and diabetics given topical EEB (174.83±49.00 U/L and 116.13±38.17 U/L), oral EEB (380.65±259.60 U/L and 195.23±98.50 U/L) and combination (478.35±335.25 U/L and 360.85±239.55 U/L) did not show any significant difference (P>0.05) (Table 2).

Liver necrosis could occur in STZ-induced diabetic rats and might lead to increased SGPT and SGOT activity in plasma. The enzyme activity was increased due to liver damage that caused leak of enzymes from the liver cell cytosol into the bloodstream (Ramachandran *et al*., 2012; Mohan *et al*., 2013). Several studies showed that changes in liver cell membranes lead to the release of intracellular enzymes into the extracellular space. Damage to these cells results in increased permeability so that cytosolic isoenzymes could came out into the liver sinusoid and peripheral blood circulation (Salih *et al*., 2012). In this study, EEB administration did not have a marked effect on SGPT and SGOT levels of diabetic rats.

Ureum and Creatinine Level

Ureum level of diabetic control rats (107.23±45.32 mg/dL) was significantly higher than non-diabetic control rats (31.95±4.83 mg/dL) (P<0.05), while ureum level of diabetic control rats (107.23±45.32 mg/dL) and diabetic rats given topical EEB (95.65±48.25 mg/dL), oral EEB (100.80±19.06 mg/dL), or its combination (115.15±27.88 mg/dL) showed no significant differences (P>0.05) (Table 3).

Creatinine level of diabetic control rats (0.31±0.04 mg/dL) showed no significant differences (P>0.05) compared to non-diabetic control rats (0.24±0.02 mg/dL). However, creatinine level tends to be higher in

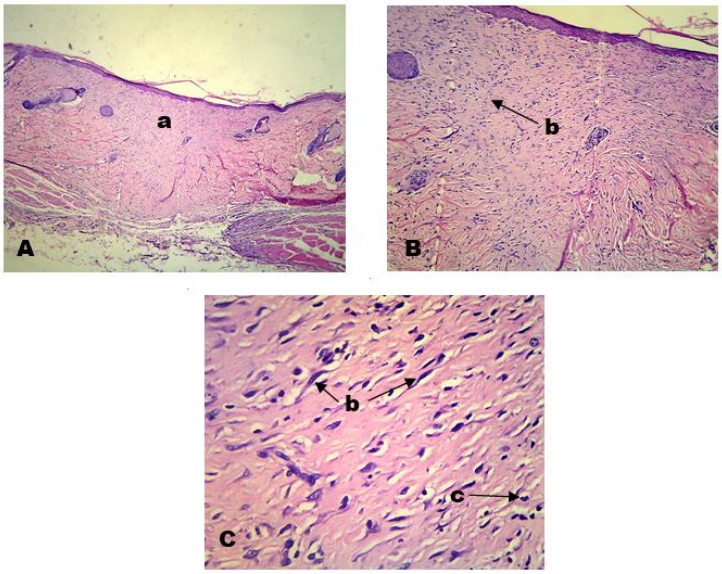

Figure 1. Skin histopathology of non-diabetic control rats on day 14 after being wounded at dermis layer. a= Region of excision wound which had closed, b= Existence of fibroblast proliferation, c= Few leukocyte infiltration (HE, A= 40x, B= 100x, C= 400x)

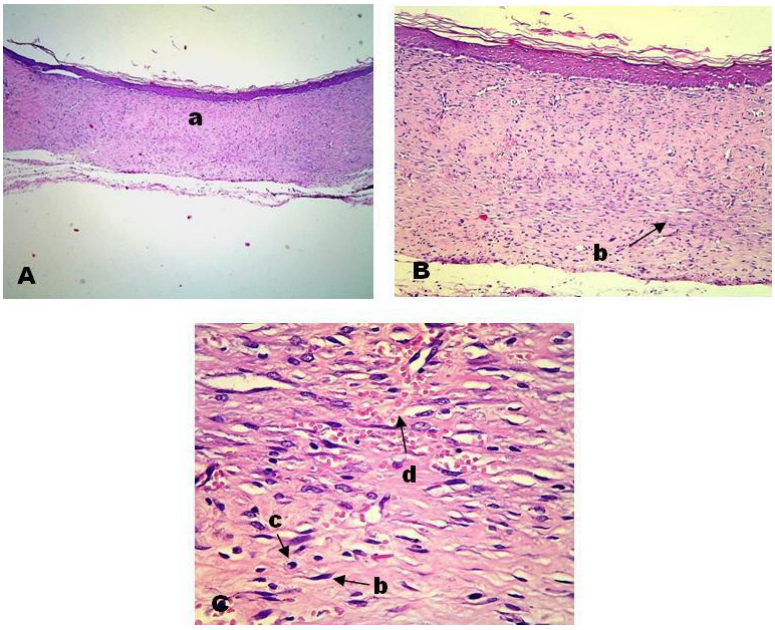

Figure 2. Skin histopathology of diabetic control rats on day 14 after being wounded at dermis layer. a= Region of excision wound which had closed, b= Existence of fibroblast proliferation, c= Leukocyte infiltration, d= Hemorrhage (HE, A= 40x, B= 100x, C= 400x)

diabetic control rats. Creatinine level of diabetic control rats (0.31±0.04 mg/dL) showed no significant differences (P>0.05) if compared to either diabetic rats given topical EEB (0.28±0.08 mg/dL) or combined with oral EEB (0.27±0.05 mg/dL), yet it showed significant differences (P<0.05) with diabetic rats given oral EEB only (0.18±0.04 mg/dL) (Table 3).

Elevated ureum and creatinine level is significant marker for diabetic nephropathy in STZ injected rats. High level abnormality of ureum and creatinine is related to renal function disturbance. Ureum level elevation of diabetic rats is related with high protein catabolism. Metabolic abnormality is shown in uncontrolled diabetes causing gluconeogenesis and urea production (de Almeida *et al*., 2012). Hyperglycemia is one of the main causes of progressive renal failure. Elevated ureum and creatinine of diabetic rats indicate the process of progressive renal failure (Shrestha *et al.,* 2008). Balasubramanian *et al*. (2014) stated that oxidative stress might be happened in diabetic nephropathy caused by elevation of ROS production and reduction of antioxidant defenses.

Ureum level of diabetic rats given either topical or oral EEB tend to be lower than diabetic control rats, while the administration of 300 mg/kg oral EEB could significantly lower the level of creatinine in diabetic rats. This was in accordance with the study of Sukandar *et al*. (2010) stated that ethanol extract of binahong leaves with the dosage of 50, 100, and 200 mg/kg could improve renal function of female rats by reducing blood creatinine level significantly and improving renal cells.

Skin Histopathology

Examination result of skin histopathology in all intervention groups showed that all excision wounds had been closed in day 14. In the dermal skin layer of all intervention rats, fibroblast proliferation and leukocytes infiltration could be found (Figure 1), yet there was hemorrhage in dermal layer of diabetic rats (Figure 2). Administration of EEB in the skin of diabetic rats showed existence of leukocyte infiltration along with reduction of hemorrhagic in dermal layer (Figure 3).

Leukocyte number in wounded skin tissue of diabetic control rats (36.88±17.30) was significantly higher (P<0.05) than non-diabetic control rats (16.71±5.82), while diabetic control rats (36.88±17.30) was significantly higher (P<0.05) than diabetic rats given topical EEB (15.67±2.34), oral EEB (23.17±4.67) or the combination (20.84±3.83) (Table 4). Based on that result, topical EEB, oral EEB, or the combination, could reduce total leukocyte in wounded skin tissue.

Wound healing of diabetic patient requires longer time than non-diabetic patient. Diabetes has several effects on wound healing such as disturbance of cellular proliferation, increase apoptosis of endothelial cell, disturbance of new vessel growth, decrease of collagen deposition in wound side, and disturbance of growth factors expression (Waugh dan Sherratt, 2006). High level of blood glucose is also related to cellular morphological changes, decrease of keratinocyte proliferation and differentiation (Tsourdi *et al*., 2013), macrophage stimulation to increase production of pro inflammatory cytokines such as IL-1β, IL-6, IL-12, IL-18, TNF α and IFN-γ (Wen *et al*., 2006). Mc Lennan *et al*. (2006) reported that diabetic wound healing is related to delay in formation of mature granulation tissue and decrease of wound tensile strength. Several pro inflammatory cytokines, such as TNF β and IL-6, could be increased in cases of chronic ulceration and burn injury. Prolonged inflammation process is associated with increase of neutrophil infiltration and protease activity. Beside, decrease of growth factors which are responsible for tissue repair, such as PDGF and TGF β, could also happen in diabetic cases.

O'Brien *et al.* (2006) as cited in Acosta *et al.* (2010) stated that phagocytic ability of macrophage in diabetic rats was lower and less efficient than non-diabetic rats. Diabetic patient was more prone to wound infection and excessive inflammation due to increase of pro inflammatory cytokines such as TNF α and IL-6 (Borst, 2004). Similar statement was reported by Xu *et al*. (2013) which found that the increase of pro inflammatory cytokines (TNF α and IL-6) and decrease of anti-inflammatory cytokine (IL-10) could be happened in diabetic animal model than in non-diabetic animal. Research data on diabetic rats also showed expression of macrophage inflammatory protein 2 (MIP-2) and macrophage chemoattractant protein 1 (MCP-1) related to the increase of neutrophil and macrophage infiltration to the wound (Wetzler *et al.*,

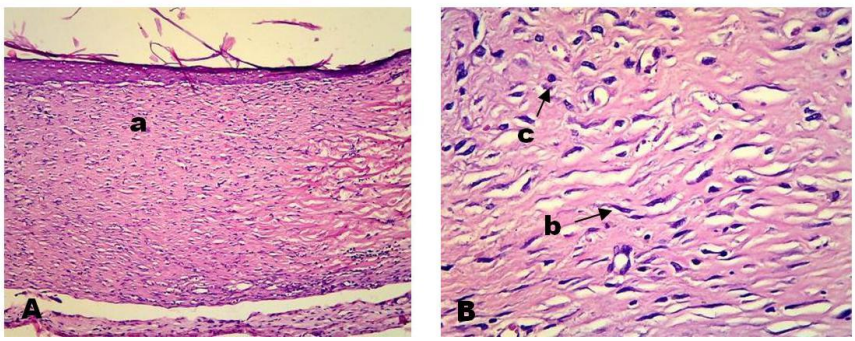

Figure 3. Skin histopathology of diabetic control rats given topical 50% EEB on day 14 after being wounded and lesser hemorrhage at dermis layer. a= Region of excision wound which had closed, b= Existence of fibroblast proliferation, c= Leukocyte infiltration, lesser hemorrhage at dermis layer (HE, A= 40x, B=100x, C= 400x)

2000). Prolonged neutrophil infiltration is associated which excessive production of elastase, ROS, and reactive nitrogen species (RNS) in the wound, which all of them have cytotoxic effect and pro degradative potential (Schonfelder *et al.*, 2005).

Excision wound of diabetic control rats in this study showed fibroblast proliferation, leukocyte infiltration, and hemorrhage in dermal layer. Topical administration of EEB in the excision wound of diabetic rats could reduce hemorrhage in dermal layer. Number of tissue leukocyte in diabetic rats given topical EEB, oral EEB, or the combination of both was significantly lower than diabetic control rats. Miladiyah and Prabowo (2012) reported that wound healing in rats given topical application of 20% and 40% binahong leaves ethanol extract showed better results than control rats given povidone iodine.

Diabetic wound healing is better when given EEB in topical or oral route. This could be happened due to substances contained in binahong plants such as saponin, alkaloid, flavonoid, polyphenol and mono polysaccharides (Astuti *et al*., 2011; Miladiyah and Prabowo, 2012). Ratna (2012) reported that binahong contained high level of antioxidant (flavonoid) as much as 9.614%. Miladiyah and Prabowo (2012) also explained that saponin could stimulate production of type 1 collagen having essential role in wound closure and epithelization. Flavonoid inhibits lipid peroxidation process and being responsible in free radical scavenging so that it prevents cell necrosis and increase vascularization in wound side. Inhibition of lipid peroxidation could increase viability of collagen fibril by increasing vascularization, preventing cellular disruption, and increasing synthesis of DNA. Polyphenol also contains antioxidant properties involved in wound healing by inhibiting lipid peroxidation process. The use of antioxidant in wound healing is very significant due to its roles in preventing free radicals on cell proliferation process, suppression of inflammation and tissue contraction.

CONCLUSION

Administration of 50% ethanol extracts of binahong leaves topically or 300 mg/kg EEB orally improved wound healing in diabetic rats.

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