Brotowali Extract (Tinospora Crispa) for Oral Traumatic Ulcer in Diabetes Mellitus Wistar Rat

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Abstract

One of the diabetes complication is delayed wound healing in oral traumatic ulcer. Tinospora crispa contains flavonoid and terpenoid which can help to control blood glucose level and accelerate wound healing.

The aim of this study is to analyze brotowali (Tinospora crispa) extract potential in controlling blood glucose level and accelerating ulcer healing process. The extract was made with oven-dried method at 50°C and macerated with ethanol 80% (1:10 w/v). The rats were divided into 3 groups, control group I (K1) normal Wistar Rat with traumatic ulcer, control group II (K2) diabetes mellitus wistar rat with traumatic ulcer, and treatment group (P) diabetes mellitus wistar rat with traumatic ulcer treated with 250 mg/kg tinospora crispa extract once a day. Sample were euthanized on 3rd, 5th, and, 7th day after traumatic ulcer was made then a histopathology preparation was made to count fibroblast cell. Blood glucose level measurement was conducted on 3rd, 5th, 7th, and 14th after traumatic ulcer was made.

Brotowali extract can affect blood glucose levels but make no significant difference and affect the number of fibroblasts in traumatic ulcers healing diabetes mellitus Wistar rats with significant difference.

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Introduction

Diabetes mellitus defined as a multi factorial disease or chronic metabolism disorder, characterized by elevated blood glucose level, carbohydrate, lipids and protein metabolism disturbance in consequence of insulin insufficiency. Insulin insufficiency may be caused by disturbance or insufficient insulin production by beta Langerhans cells of pancreas[1].

Indonesia is the fourth country that has the highest diabetes mellitus prevalence[2]. According to report from Basic Health Research (Riskesdas) 2013, the prevalence of diabetes mellitus in Indonesia is 6,9% or equivalent to 12,1 million patients[3]. According to research by Diabcare, type 2 uncontrolled diabetes mellitus patient reached 47,2% with plasma glucose level >130mg/dL[4].

Diabetes Mellitus have various complications related to dentistry, one of them is traumatic ulcer[5,6]. Traumatic ulcer is single mucosa ulcer that can be caused by mechanic, thermal, chemical, or direct physical trauma to the mucosa[7,8]. Traumatic ulcers were more common in diabetes mellitus patient than in normal person. This may be caused by wound healing impairment[9]. In patients with diabetes mellitus, many aspects are disrupted during wound healing process, such as inflammatory response dysfunction, reduced granulation tissue formation, angiogenesis disorders, and increased cell apoptosis[10].

Brotowali is widely known as traditional medicine as antidiabetic agent and antioxidant that accelerate wound healing process[11]. Brotowali stem contains flavonoids which have antioxidant effect better than ascorbic acid[12]. Flavonoid as antioxidant can bind free radical and decrease ROS level which is advantageous for wound healing process[13]. Low ROS level will

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lead to decrease of excessive cell apoptosis[14]. Moreover, flavonoid ability to activate macrophage has been proven[12]. Macrophage plays an important role in wound healing as a resource of various growth factors, such as *Transforming Growth Factor* (TGF-β*), Platelet-Derived Growth Factor* (PDGF*) and Fibroblast Growth Factor* (FGF*)* which play role in fibroblast proliferation[15]. Brotowali stem also contains terpenoid that contains borapetosid, with ability to enhance insulin receptor fosforilation, and increase protein kinase B and GLUT2 expression in hepar. This may lead to increase of peripheral utilization of glucose and decrease of gluconeogenesis in hepar[16,17].

Hyperglycemic condition in diabetic patient stimulate excessive glucose in blood to enter sorbitol pathway that followed by elevated ROS formation. Due to antihyperglycemic activity of brotowali, sorbitol pathway stimulation does not occur, so that ROS formation can be reduced, and prevent fibroblas apoptosis caused by ROS[18]. This research is conducted to analyze the effect of brotowali extract on blood glucose level and the number of fibroblasts in traumatic ulcer healing process in Wistar rats with diabetes mellitus.

Materials and methods

This was an experimental laboratory with post test only control group design. This study was approved by Ethical Comitee of Faculty of Dental Medicine Universitas Airlangga (NO 59/KKEPK.FKG/V/2016).

Brotowali Plant Extraction

Fresh specimen of brotowali stem washed and cut into small pieces and dried with oven in of 50^{o}C temperature. Dry specimenwas made into powder and macerated with ethanol 80% (1:10 w/v) for 24 hours. The extract was filtered, dregs and filtrate were subsequently separated. The dregs were macerated again with new ethanol, this procedure is repeated until TLC (Thin Layer Chromatography) test with H_2SO_4 is not showing pink color. Then the dregs and the filtrate from first to the last day is collected and evaporated with a rotary evaporator to remove the solvent. Extracts were stored at temperature of -20^{o}C [19].

Preparation of Experimental Animals

Male Wistar rats 150-200g weights were collected in the same cage with a room temperature of 25±2°C and given food pellets and a standard drink distilled water ad libitum for 7 days prior the experiment[19]. Animals were divided into 3 groups, the control group 1 (K1), control 2 (K2), and treatment (P). Each group contains 8 experimental animals.

Diabetes Mellitus Induction with Alloxan

For rats in groups K2 and P1, alloxan (120 mg/kg) dissolved in 0.05 M citrate buffer pH 3 was used to induce experimental diabetes. K1 group (control group) were injected with sterile saline solution 72 hours after injection, blood from lateral vein was drawn to determine the blood glucose levels by “on call plus” ACON Sandiago USA. Animals stated diabetes when blood glucose levels ≥200 mg/dL after 72 hours post-induction of alloxan[20,21].

Wistar rats in all treatment groups were anesthetized using injection of ketamine (Biosynth, Switzerland) (60mg/kg) and xylazine (PT Merck Chemicals & Life Sciences. Jakarta) (60mg/kg) intraperitoneal[22]. The buccal mucosa is sterilized with a swab moistened with a 0.12% chlorhexidine digluconate. Buccal mucosa ulceration created by wounding the mucosa using a scalpel blade no. 15 with a cross-section diameter wound 8 mm[23]. Brotowali extract administered per oral (250 mg/kg) once a day for the treatment group P1 after traumatic ulcer is formed. Extracts brotowali given until day 3, 5, 7 and 14.

Euthanasia Procedure at Day 3, 5, 7, and 14 Post Traumatic Ulcer Creation

Prior sacrificed, blood glucose levels of experimental animals in all groups are checked using a glucometer. Then euthanasia of experimental animals is performed with ketamine injection of 180mg/kg and xylazine 180mg/kg. Buccal mucosa tissue removal was done in all groups and put in formalin prior to the procedure of making preparat. The number of fibroblasts cells is determined by observation of HE preparations under light microscope (Olympus, Tokyo Jepang) with 400x magnification. Fibroblasts cell calculation using ocular graticulae tool mounted on the lens on a microscope with a visual field technique conducted on three field[24].

Results

Level of blood glucose on day 3 in control I group is significantly lower than in

control II group and treatment group ($p<\alpha = 0.05$). On day 5, blood glucose decreased in control 1group, increased in control group 2 and decreased in treatment group ($p<\alpha = 0.05$). On the 7th day blood glucose levels decreased in control 1group, increased in control 2 group and treatment groups remained the same ($p<\alpha = 0.05$). On the 14th day blood glucose level increased in control 1group and decreased in control 2group and treatment group (Figure 1).

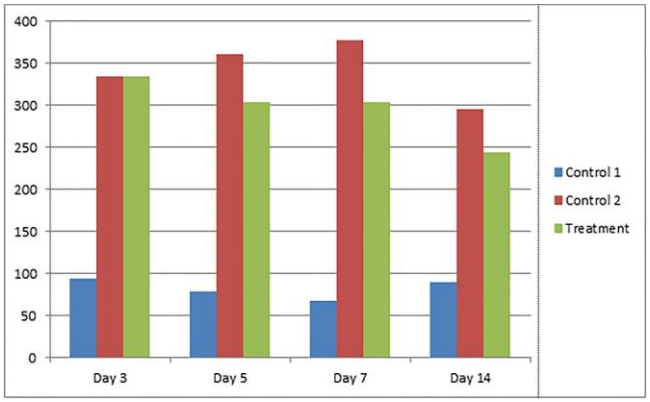

Figure 1. Blood glucose level diagram at day 3, 5, 7 and 14.

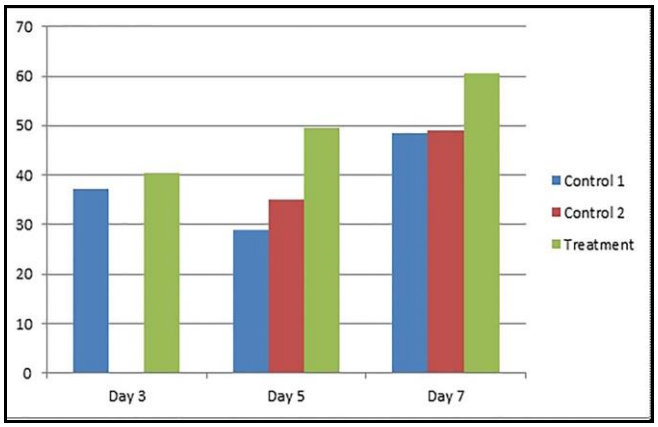

Figure 2. Average number of fibroblasts on day 3, 5, and 7.

On day 3, there is no significant difference ($p> \alpha = 0.05$) between the number of fibroblast cells control in control 1 group than in treatment group. On day 5, the number of fibroblasts in control 1group slightly lower while in treatment group is higher ($p> \alpha = 0.05$). On day 7, the number of fibroblasts increased in all groups ($p> \alpha = 0.05$) (Figure 2). The number of fibroblasts in treatment group continues to increase and has the highest average among each group (Figure 3).

Based on the result of fibroblast cell count, there are no significant difference between each group in day 3 (Figure 3). The number of Fibroblast is increased in day 5, and the number of fibroblast in treatment group is significantly higher than in control I and 2 group ($p> \alpha = 0.05$) (Figure 4). On day 7, the number of fibroblast cell increase in all group, with the highest number of fibroblasts are found in treatment group ($p> \alpha = 0.05$) (Figure 5).

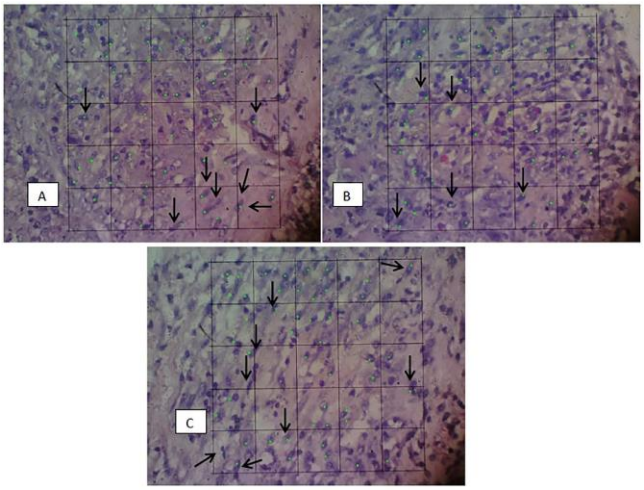

Figure 3. Fibroblast cells (arrows) on day 3. (A) control 1 group, (B) control 2 group and (C) treatment groups on day 3 with HE stainning and 400x magnification.

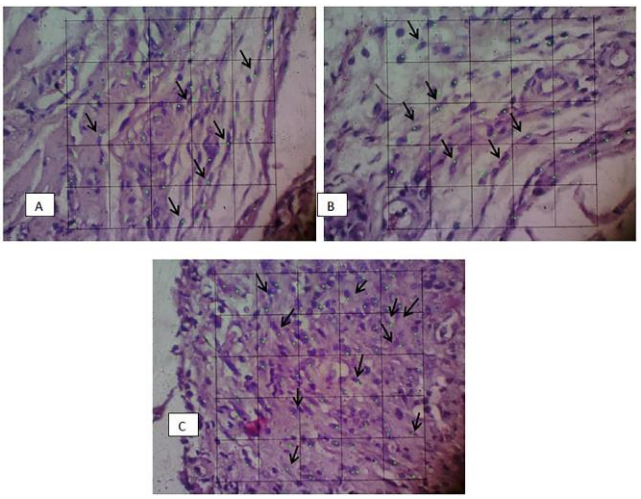

Figure 4. Fibroblast cells (arrows) on day 5. (A) control 1 group, (B) control 2 group and (C) treatment groups on day 5 with HE stainning and 400x magnification.

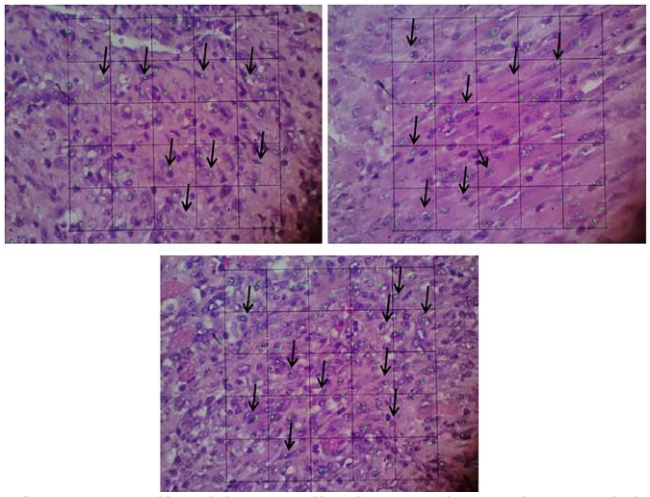

Figure 5. Fibroblast cells (arrows) on day 7. (A) control 1 group, (B) control 2 group and (C) treatment groups on day 7 with HE stainning and 400x magnification.

Discussion

The average blood glucose level in treatment group decreased in each observation days and lower than blood glucose in control 2 group but the difference was not significant. This means that the blood glucose in treatment groups had not back to normal. This result is contrary with the previous research that said there was significant decrease in blood glucose by 10% at day 14 after the administration brotowali extract at a dose of 250 mg/kg[19]. Other studies suggested that there were significant differences on blood glucose levels on day 7 and 14 after administration brotowali extract at a dose of 250 mg/kg[25].

Blood glucose levels were not significantly decreased may be caused by the duration of extract administration. Brotowali has ability to reduce blood glucose because contains terpenoids that consists of borapetosid A which can enhances insulin receptor phosphorylation and protein kinase B as well as increases the expression of GLUT2 in liver. This may lead to increase of peripheral utilization of glucose and decrease of gluconeogenesis in heparsoglucose levels in blood will decrease[17]. To obtain significant results, duration of 60 days is required[26]. Previous study stated no significant difference in blood glucose after administration brotowali extract[27]. These different results might be attributed to the stability of the material, so it is important to note the time between manufacture of extracts and time of treatment[28]. Monitoring the concentration of active ingredients is important because it also affects the quality, effectiveness and life of natural medicines during storage[29]. In this study, the time between manufacture of extracts and treatment is a month. This may lead to the stability of the extract brotowali reduced.

In wound healing process, fibroblast cells maintain structural integrity of connective tissue by secreting extracellular matrix precursor such as collagen, reticulum, and elastin continuously[30]. Catechins in brotowali can accelerate wound healing process, through the intermediary role of macrophages that stimulates growth factors to increase fibroblasts proliferation. Macrophages phagocytes bacteria that can interfere wound healing process in patients with diabetes mellitus and eliminate tissue debris, then release cytokines and growth factors such as PDGF, TGF-β and FGF. Those cytokine products stimulate cell migration and fibroblasts proliferation, as well as production and modulation of extracellular matrix[24].

Catechins also increase the activity of superoxide dismutase and catalase. Superoxide dismutase is an enzyme that catalyzes superoxide into oxygen and hydrogen peroxide. Catalase is an enzyme that catalyzes hydrogen peroxide to water (H_2O). Other content of brotowali extract is luteolin that binds superoxide radical in the body and with catechins make antioxidant effects that reduce ROS level in the body. This leads to decrease of fibroblasts apoptosis rate[10].

Wound healing process can be observed histologically by counting the number of fibroblast cells to determine cell proliferation. From the research data, there is no significant difference in the number of fibroblasts in control I group and treatment groupon day 3. This is because day 3 is an early phase of traumatic ulcer healing[23]. Proliferation in fibroblast cell count began to increase on day 5 and day 7. This study used Wistar rats which have healing time faster than humans and synthesis of collagen by fibroblasts begin relatively early[31]. Synthesis of collagen by fibroblasts begin relatively early in day 3-5 and continue until a few weeks depending on the size of the wound[32]. From the data results, the number of fibroblasts in the control 1 and 2 group is less than the treatment group. The treatment group had an average number of fibroblasts at

most in all the groups. On the 7th day fibroblast cell number continues to increase and has the highest average when compared with the control group. Supposedly at day 7 the proliferation of fibroblasts was decreased for the formation of collagen fibers. This indicates proliferation of fibroblasts still high due tobrotowali extract. Excessive fibroblasts proliferation may cause a build up of collagen so that the wound will heal with scars or fibrous tissue[32].

The number of fibroblasts which remained high at day 7 may be caused by circadian rhythms. Wistar rats are nocturnal rodents which active at night and relatively inactive during day[31]. Diabetes induction, extract administration and euthanasia procedures which is not suitable with rats' circadian rhythm could affect the research results. Circadian rhythm affects the immune system. One of hormones associated with circadian rhythm is cortisol, that also regulate inflammation process[33]. In this research, all procedures performed at 8-12 am. There was no cortisol activity as anti-inflammatory agent during that time. Lack of cortisol lead to lack of ability to prevent severe chronic inflammation. As a result, immune system goes out of control. Low cortisol levelmay increase the production of pro-inflammatory cytokines, which lead to over-activation of immune system and inflammation[33]. Low cortisol level may lead to the absence of a regulatory function growth factors secretion that results in excessive secretion of growth factor. In this research, high level of fibroblasts proliferation is caused by over-activation of fibroblasts during proliferation phase of healing process in the morning[10].

Conclusions

This research showed that administration of brotowali (*Tinospora crispa*) extract dose of 250 mg/kg for 14 days can reduce blood glucose wistar rats with diabetes mellitus and increase the number of fibroblasts on day 3, 5 and 7 of oral traumatic ulcer healing on diabetes mellitus Wistar rats.

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Declaration of Interest

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