

Antihyperglycemic and diabetic wound healing activity of *smallanthus sonchifolius* leaves extract

Rina Herowati*, Alip D.S. Saputri, Tri Wijayanti, and Gunawan P. Widodo

Universitas Setia Budi, Faculty of Pharmacy, Jalan Letjen Sutoyo, Solo 57127, Indonesia

Abstract. Diabetic patient tend to suffer from lower extremity complication that contribute to the occurrence of diabetic foot ulcer. *Smallanthus sonchifolius* or yacon leaves extract has been used as an alternative medicine for diabetes and also showed antibacterial activity. This research aims to obtain the antihyperglycemic and diabetic wound healing activity of yacon leaves extract. Diabetic condition of rat was induced by streptozotocin (45 mg/kg bw) and nicotinamide (110 mg/kg bw). Rats were then allowed to develop diabetes for 21 days. A biopsy punch then was used to create a wound. Yacon leaves extract were administered by oral and topical for 14 day. The serum glucose level and diameter of the wound were measured every week, and histopatology study of the skin was conducted at the end of the study. Oral administration of yacon leaves extract (150 mg/kg bw) together with topical administration showed antihyperglycemic as well as diabetic wound healing activities comparable to the reference drugs (glibenclamide oral and nebacetin cream).

1 Introduction

Diabetes is a serious chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin. More than 400 million adults were living with diabetes in 2014. When diabetes is not well managed, complications develop that threaten health and endanger life. stroke. Such damage can result in reduced blood flow, which combined with neuropathy in the feet will increases the risk of foot ulcers, infection and lower extremity amputation. Rates of amputation in populations with diagnosed diabetes are typically 10 to 20 times those of non-diabetic populations, and over the past decade have ranged from 1.5 to 3.5 events per 1000 persons per year in populations with diagnosed diabetes [1].

Wound healing is started as normal biological process including hemostatis, inflammation, proliferation and remodeling. All the healing processes must occurs in suitable order for complete healing, but sometimes complex process of wound healing can be delayed or failed by unwanted factor as presence of free radical or microbial infection. Usually these open wounds become rapidly colonized by microorganism and often requires antimicrobial therapy. Delayed wound healing and chronic wound management in diabetics is one of health problem due to the social and economic impact. One of the key problem is high blood glucose level. High blood glucose level inhibits wound healing process associated with prolonged inflammatory phase [2].

The use of plant extract or traditional medicine with known antihyperglycemic as well as antimicrobial properties can be of great opportunity in treatments of

diabetic wound. One of these medicinal herbs is *Smallanthus sonchifolius*, also is known as yacon or insulin leaves [3-4].

Yacon leaves extract attenuates hyperglycemia, oxidative stress and inflammation in diabetic rats [5]. A decoction of yacon leaves reduced blood glucose level in streptozotocin induced diabetic rats and increased the insulin concentration [6].

Yacon leaves rich in caffeic, chlorogenic and three dicaffeoilquinic acids. These compounds are responsible for the hypoglycemic activity of yacon leaves, however their mechanisms of action are still unknown [7] This study aims to evaluate the antihyperglycemic and diabetic wound healing activity of *S. sonchifolius* leaves extract on STZ-NA induced diabetic rats.

2 Material And Methods

2.1 Animals

This study was approved by the Health Research Ethics Committee of the Dr. Moewardi General Hospital and School of Medicine Sebelas Maret University (No. 1.090/XII/HREC/2017). Health male Wistars rats, weight of 180 to 220 g, were used in this research. The animals were adapted for 1 weeks, maintained with free access to food and water and kept at room temperature.

* Corresponding author: rinagunawan53@gmail.com

2.2 Preparation of the extract

The leaves of the plant were washed and properly cleaned by distilled water. The leaves then was dried and powdered, and subjected to maceration at room temperature in ethanol for five days. The solvent was evaporated to dryness using evaporator in vacuum system [8].

2.3 Phytochemical screening

Phytochemical screening of test compounds was conducted based on the standard procedures (table 1). The extracts were subjected to phytochemical tests for determination the secondary metabolites i.e. terpenoids, alkaloids, flavonoids, saponin, as well as tannins and phenolic compounds [9].

Table 1. Phytochemical screening methods.

Secondary metabolite	Test method	Positive result
Terpenoid	Liebermann-Buchard	An array of colour change
Flavonoid	Alkaline reagent	Yellow fluorescence
Saponin	Foam test	A two cm layer of foam
Tannin and phenolic compound	Ferric chloride	A dark green colour

Liebermann-Burchard's test: The extract (50 mg) was dissolved in of 2 ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid were added slowly along the sides of the test tube. Alkaline reagent test: An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Ferric Chloride test: The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution were added. Foam test: The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken for 15 minutes.

2.4 Induction of diabetes

Animals were divided into 8 groups, 5 rats each group. The animals in group I were non-diabetic, and in groups II to VIII were diabetic (table 2). The rats in group II-VIII were fasted overnight before intra peritoneal administration of STZ (45 mg/kg) - NA (110 mg/kg). After 5 days of STZ injection, blood glucose level was estimated using glucometer. Rats with blood glucose levels more than 250 mg/dl were considered as diabetic and used for the study. Rats were then allowed to develop diabetes for 21 days [10].

2.5 Wound induction and treatment

All the rats were anaesthetised and shaved on the back. A biopsy punch diameter of 4 mm was used to create a 2 mm

depth wound. After wound induction, the administration of oral and/or topical drugs and extract were conducted every day until 14 days. Blood glucose level and diameter of wound area were measured on day 7 and 14 after the treatment. On day 15 the animals were sacrificed under anesthesia, and the skin specimens of all tested groups were collected for histopathological studies. Hematoxylin eosin (HE) staining methods were used for evaluation of histopathological changes of wound site. The tissue samples were fixed in formalin and embedded in paraffin. The the tissue sections with 5 μ m thickness were taken and stained with HE [2].

Table 2. Experimental design of animals group.

No	Group	Administration
I	Non-diabetic group	CMC Na 0.5% (5 ml/kg) orally
II	Diabetic control	CMC Na 0.5% orally and basis of cream topically
III	Oral control drug	Glibenclamide 0.45 mg/kg
IV	Topical control drug	Nebacetin cream
V	Oral & topical control drug	Glibenclamide 0.45 mg/kg and Nebacetin cream
VI	Oral extract	Extract yacon leaf 150 mg/kg orally
VII	Topical extract	Extract yacon leaf topically
VIII	Oral and topical extract	Extract yacon leaf 150 mg/kg orally & topically

3 Results And Discussion

3.1 Phytochemical analysis

Phytochemical analysis result revealed the constituent of terpenoid, alkaloid, flavonoid, saponin, as well as tannin and phenolic compound in yacon leaves extract. This results was in line with previous research results. Some terpenoids have been successfully isolated from yacon leaves, i.e. sonchifolin, polymatin B, uvedalin, enhydrin, fluctuanin, ent-kaurenoic acid and its angeloyloxide derivatives, ent-kaurane-tetrols and smaditerpenic acids. *S. sonchifolius* leaves are rich in phenolic compounds, such as gallic, chlorogenic, caffeic and ferulic acids; as well as the flavonoid rutin, myricetin, kaempferol and quercetin [11].

3.2 Antihyperglycemic activity

Measurement of blood glucose level was conducted after the rats were developed diabetes for 21 days (day 0). The occurrence of DM in the diabetic groups (group II-VIII) on 21 days after induced STZ-NA was characterized by the high blood glucose levels (above 200 mg/dL). Intra peritoneal administration of STZ induced the damage of pancreatic β cells, resulted the reduction of insulin production and increasing the blood glucose levels. STZ

enters the pancreatic β cells through the GLUT-2 (glucose transporter-2) causing DNA alkylation. DNA damage leads to inhibition of insulin synthesis and secretion. NA administration aims to decrease the toxic effects caused by STZ, so reduces the severe damage of pancreatic β cells and inhibit the development of type-1 diabetes [10].

Antihyperglycemic activity of the reference drug and yacon leaves extract after 7 and 14 days administration was presented in table 3. Oral administration of glibenclamide and combination of oral glibenclamide-topical nebacetine cream decreased the blood glucose level. However topical administration of nebacetine failed to decrease the blood glucose level. Sulfonylureas, such as glibenclamide, show potent antihyperglycemic activity by enhance the secretion of insulin. Sulfonylureas bind to a specific sulfonylurea receptor (SUR) on pancreatic β cells, increase the intracellular Ca^{2+} and cause translocation of secretory granules of insulin to the cell surface and exocytosis of the granule of insulin [12].

Table 3. Antihyperglycemic effect of yacon leaves extract.

Group	Blood glucose level (mg/dL)		
	Day 0	Day 7	Day 14
I	81.80 ± 2.36	81.09 ± 2.42	81.73 ± 2.55 ^{b,c}
II	213.10 ± 7.37	214.17 ± 7.91	215.64 ± 7.19 ^{a,c}
III	210.13 ± 3.64	184.03 ± 1.83	149.95 ± 2.45 ^{a,b}
IV	210.73 ± 8.29	211.61 ± 8.93	213.09 ± 8.44 ^{a,b}
V	212.97 ± 6.89	183.71 ± 1.89	148.64 ± 1.73 ^{a,b,c}
VI	214.27 ± 7.44	183.71 ± 1.89	182.36 ± 1.98 ^{a,b,c}
VII	214.60 ± 7.28	215.32 ± 1.89	217.33 ± 3.89 ^{a,b,c}
VIII	213.43 ± 4.06	187.48 ± 3.91	161.94 ± 2.37 ^{a,b,c}

a : different to normal control ($p < 0.05$)

b : different to diabetic control ($p < 0.05$)

c : different to drug control ($p < 0.05$)

Administration of yacon leaves extract showed the similar antihyperglycemic activity profile. This results was in line with previous research results. Baroni et al. reported that ethanol extract of yacon leaves dose of 400 mg/kg for 14 days, significantly reduced the blood glucose levels of the diabetic rats. However this effect was not observed when the crude aqueous extracts were administered in the same dosage. [8] Lee et al. reported that yacon leaves improve the fasting blood glucose level and glucose tolerance, through increasing the concentration of pancreatic C-peptide. C-peptides are substances released by pancreatic β cells during the breakdown of proinsulin into insulin. Therefore, this molecule can be used to determine the function of pancreatic β cells. Increased concentrations of C-peptide by insulin-leaf administration correlated to the increasing of secretion insulin by pancreatic β cells [13].

Some phytochemicals constituents of yacon leaves extract such as flavonoids and polyphenols play a role in significant decrease of blood glucose level. Yacon leaves extract significantly increased the antioxidant status and endogenous antioxidant activities as well as decreased the markers of lipid peroxidation and proinflammatory cytokine in soleus muscle in diabetic rats. These results indicated that yacon leaves have free radical scavenging activity and promoting decrease of oxidative stress under diabetic conditions. The sesquiterpene lactone enhydrin, diterpene ent-kaurenoic acid, caffeic, chlorogenic and three dicaffeoilquinic acids were suggested responsible for the antihyperglycemic activity of yacon leaves. Smallanthaditerpenic acids (A – D) also contributed to this activity by inhibition of α -glucosidase activity.[14-15]

3.3 Macroscopic evaluation of wound healing

The wound healing activity of the yacon leaves extract was evaluated on rats based on the diameter of the wound. Photographs of the wound were performed at the wound creation day, that is, 0 and on days 7 and 14. Figure 1 showed the wound on days 14. in all groups. The measurements of the progress of wound healing induced by oral and/or topical administration of the extract and reference drug are shown in table 4.

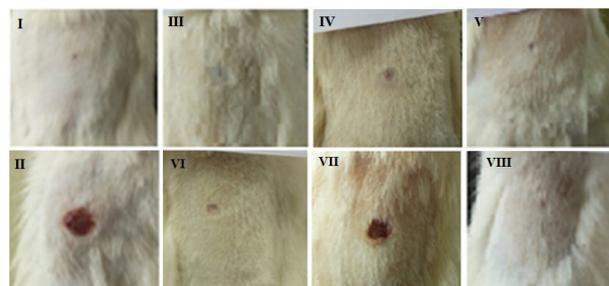


Fig. 1. Photographs of macroscopic appearances of wound.

The wound contraction of normal control was achieved on day 7, and the diameter of the wound reached 1 mm. However, in diabetic control, the wounds were not contracted, the diameter was more than 5 mm after 14 days. In addition of oral and oral-topical drug that showed the contraction of the wound, oral and oral-topical extract also showed the wound healing activity.

After injury, the skin wound healing process occurs immediately, consist of inflammation, proliferation, and maturation phases. Inflammation is defense mechanism of the tissue to protect from microbial contamination. However, prolonged inflammation phase causes delaying of healing process. [16] Anti-inflammatory activity of yacon leaves extract plays a role for shorten the healing period.

There was significant increase in wound area in diabetic wound control when compared to the normal wound control. However, orally and topically treated yacon leaves extract showed significant decrease in wound area when compared with diabetic wound control. The macroscopic findings revealed the improvement of the wound healing process and the reduction in the wound

size compared to the diabetic, drug control and healthy reference groups.

Oral and oral-topical administration of yacon leaves extract showed wound healing activity, however topical administration failed to reduce the diameter of the wound. Yacon leaves contain some constituents that have antibacterial activity, i.e. sonchifolin, fluctuanin, uvedalin, enhydrin, Ent-kaurenoic acid, enhydrin, and uvedalin. [14] Yacon leaves also contain sesquiterpenelactones, which play a role in wound healing. Topical administration of yacon leaves extract reduced the inflammation by 44.1% compared to the control group [17].

Table 4. Antihyperglycemic effect of yacon leaves extract.

Group	Ø of the wound (mm)		
	Day 0	Day 7	Day 14
I	4.00±0.00	1.63±0.50	1.02±0.11 ^{b,c}
II	4.00±0.00	4.11±0.07	5.61±0.89 ^{a,c}
III	4.00±0.00	3.74±0.11	1.21±0.06 ^{a,b}
IV	4.00±0.00	3.94±0.08	1.48±0.10 ^{a,b}
V	4.00±0.00	3.56±0.06	1.27±0.20 ^{a,b}
VI	4.00±0.00	3.93±0.04	1.14±0.09 ^{a,b,c}
VII	4.00±0.00	3.96±0.04	3.24±0.32 ^{a,b,c}
VIII	4.00±0.00	3.67±0.14	1.05±0.04 ^{b,c}

a : different to normal control (p<0.05)

b : different to diabetic control (p<0.05)

c : different to drug control (p<0.05)

3.4 Microscopic evaluation of wound healing

Infection often potentiated the impairment of wound healing by DM, causes a significant amount of human morbidity and has prompted the investigation. Figure 2 showed the histopathology results.

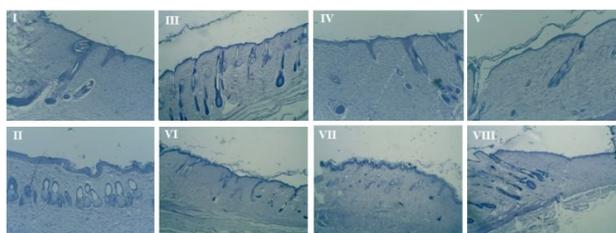


Fig. 2. Photographs of HE staining on 14th day in wound.

Superficial wounds in which the integrity of the basement membrane is preserved heal by a process of epithelization [18]. In diabetic control, the epithelium was till broken. In topical application process of epithelization have not completed yet.

Hyperglycemia is the major cause of diabetic complications and oxidative stress is one of the potential mechanisms by which hyperglycemia can result in diabetic complications. Development and progression of

diabetic complications can be inhibited by blood glucose level control [9].

4 Conclusion

S. sonchifolius or yacon leaves extract dose of 150 mg/kg oral in combination with topical administration showed antihyperglycemic activity as well as diabetic wound healing activity on STZ-NA induced diabetic rats comparable to the drug control.

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