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Research article

Wound healing activity of *Saurauia vulcani*, Korth. aqueous leaves extract evaluation on excision wound in hyperglycemia rats

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Key words: Excision wound, *Saurauia vulcani,* Korth., Oral Treatment, Hyperglycemia.

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Abstract

Objectives: This study investigated wound healing activity of oral treatment with *Saurauia vulcani,* Korth. aqueous leaves extract on excision wound in hyperglycemia male wistar rats. **Methods:** Diabetes mellitus was induced in rats by intraperitoneal injection of a single dose of streptozotocin (STZ, 55 mgkg^{-1} b.wt.). Three days after induction, full thickness excision wound were made in hyperglycemia rats and were divide in groups, each containing 5 rats. The different test group animals were treated with aqueous extract of *Saurauia vulcani,* Korth. leaves (AESVKL) 0.25 mL, 0.5 mL and 1 mL orally and compared with conventional drug Metformin for 15 days. The wound healing in hyperglycemia rats was studied by measuring blood glucose and wound healing in both control and treated groups. The means of wound area measurement between groups at different time intervals were compared using ANOVA test. **Results:** Oral treatment of AESVKL decreased blood glucose levels and on excision wounds caused the significantly faster reduction in the wound area as compared to Metformin. **Conclusions:** Findings of the present study provide a baseline data on excision wound healing and potential of *Saurauia vulcani,* Korth. Leaves to reduce glucose plasma in hyperglycemia rats and supports their traditional claim.

Introduction

Diabetes mellitus (DM) and its complications are the leading causes of death in most countries. In uncontrolled hyperglycaemia conditions can cause a variety of metabolic complications or long-term vascular complications. Diabetics are also susceptible to injury and infection of the wound which can then develop into gangrene, thus increasing cases of amputation and death after amputation. Epidemiological studies report more than a million amputations in people with diabetes [1-2].
The high blood glucose is a common factor that causes DM patients to heal wounds longer, other than that wound healing disorders in patients with DM caused by extension phase of wound healing, hemostasis, inflammation, proliferation, and remodeling and low bioavailability growth factors [3-5].
Indonesia is one of the countries with the second highest biodiversity of tropical forests in the world after Brazil [6]. One of the medicinal herbs is Saurauia vulcani, Korth. known as pirdot (Toba), cep-cepan lembu (Karo) and sopsopan (Simalungun) [7-8]. Pirdot is one of the wild plants in the forest of North Sumatra. Based on the empirical data of Pirdot leaf decoction is believed by the people of Simalungun, Toba, and Karo to have healing properties of DM [7-8], rheumatism [8], and the leaf is used to heal the wound [9]. Several in vitro and in vivo studies have shown that Saurauia vulcani, Korth. leaf extract has activity as antihyperglycemia [10-12], and antihiperlipidemia [12].
The above descriptions encourage researchers to examine the effect of AESVKL administered orally in lowering blood glucose and healing excision wounds in hyperglycemic rat.

Material and methods

Material

Diabetogenic drug Streptozotocin was obtained from Nacalai Tasque, Kyoto, Japan and analytical grades of additional compoundsand chemicals were used.

Plant collection

Saurauia vulcani, Korth. leaves were collected from Sipangan Bolon village, Girsang regency, North Sumatera province in Indonesia, and their taxonomic identification was confirmed by Herbarium Medanense (MEDA) No. 1767/MEDA/2018, Universitas Sumatera Utara, located at Medan, Indonesia.

Preparation of extract

The leaves of *Saurauia vulcani,* Korth. were shade dried to prevent the degradation of phytochemicals properties, powdered and extracted with water using infussion

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apparatus. Accurately weighed powdered *Saurauia vulcani,* Korth. (5 g) were extracted with 50 mL aquadest (1:10) at infussion apparatus for 15 minutes, measured temperature 96-98ºC. Then the aqueous extract was filtered using filter paper whatman no. 1 (pore size 11 um) and stored at 2-8ºC [13].

Preliminary phytochemical screening

phytochemical tests were performed for testing various different chemical groups present in aqueous extracts [14-16].

Experimental animals

White male wistar rats (180-200 g) were used to assess wound healing activity. The rats were kept and maintained during one week under standard laboratory conditions at room temperature condition 25-30ºC. The animal were fed with standard laboratory diet and allowed to drink water at one's pleasure. The studies were carried out in accordance with the institutional ethical guidelines based on National Guidelines on Health Research Ethics 2011 (NGHRE) indonesia. Rats were acclimatized for 1 week.

Preparation for oral administration of AESVKL

An amount of 50 ml of AESVKL was collected from infussion apparatus and devided into several concentrations: 0.25 mL, 0.5 mL, 0.75 mL and 1 mL as treatment dose in this study

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed in overnight fasted normal rats. Rats were divided into six groups (n = 4). Group I served as negative control and received orally aquadest (1 mL), groups II served as positive control and received orally Glibenclamide (0.45 mg kg^{-1} b.wt), groups III, IV, V and VI, received AESVKL orally (0.25 mL, 0.5 mL, 0.75 mL and 1 mL) respectively. After these treatments all groups received glucose (5 g kg^{-1} b.wt) orally. Blood was withdrawn from the tail vein just prior to and 30, 60, 90 and 120 min after the oral glucose administration. Blood glucose levels were measured using single touch glucometer (*EasyTouch®GCU*) [17].

Induction of experimental hyperglycemia

The rats were induced with STZ solution (55 mg kg^{-1} b.wt) i.p. and blood glucose level of the rats were measured on the 3rdday. On the 3rd day, rats that have blood glucose level higher than 200 mg/dL were separated and used as test animals.Animals with blood glucose level lower than 200 mg/dL, were induced back with STZ. If on the 3rd day, blood glucose level of the rat has been higher than 200 mg/dL, the animal was ready to be tested [18].

Making excision wounds

All rats were anesthetized with ketamin-hameln (Pt. COMBIPHAR) at dose of 70 mg/kg-b.w (i.p.). The hair on the back of each animal was shaved and sterilized with 70% alcohol. Full thickness skin wound excision of an area with diameter 2 cm and depth wound 0.3 mm was perfomed on the back of each animal. Every day the wound was monitored, cleaned with alcohol 70% to prevent contamination [19].

Antihyperglycemia and wound healing Activity of AESVKL

Antihyperglycemia and wound healing activity was performed in hyperglycemia rats with excision wounds. Rats were divided into five groups (n = 5). Group I served as negative control and received orally aquadest (1mL), groups II served as positive control and received orally Metformin (45 mg kg^{-1} b.wt), groups III, IV and V, received AESVKL orally (0.25 mL, 0.5 mL and 1 mL) respectively. Given daily treatment for 15 days. Measurement of blood glucose and wound observations were performed on the same day, on the 3rd, 6th, 9th, 12th and 15th days. Blood was withdrawn from the tail vein and were measured using single touch glucometer (*EasyTouch®GCU*). Wound observations were performed visually by measuring the diameter of the wound with a digital caliper tool and was calculated as percent reduction in wound area given in the formula below.

$$\% \text{ Wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100$$

(Healed area = original wound area – present wound area) [21].

Statistical analysis

IBM SPPSS Statistics 24.0 (SPSS, Inc., USA) was used to statistically evaluate the results. In addition, the statistical signifiance of the results has been evaluated using one-way ANOVA and Tukey's test. All the differences were considered statistically significant if $p < 0.05$.

Results and discussion

Phytochemical analysis

Phytochemical analysis showed presence of flavonoids, tannins, saponins and glycosides.

Effect of AESVKL on OGTT

Glucose challenge to normal rats increased blood glucose levels with maximum level at 30 minutes and returned to normal level at 120 minutes. The AESVKL administration improved glucose tolerance significantly ($P < 0.05$) at 60 min to 120 min compared to positive control animals. In AESVKL treated rats improved

glucose tolerance significantly (P<0.05) in a dose dependent manner (Figure 1).

Antihyperglycemia and wound healing activity of AESVKL on excision wound in hyperglycemia rats

Effect of AESVKL on blood glucose

Administration of AESVKL in hyperglycemia rats at the dose of 0.25 mL, 0.5 mL and 1 mL produced significant (P<0.05) and dose dependent fall in blood glucose levels when compared with negative control (Figure 2).

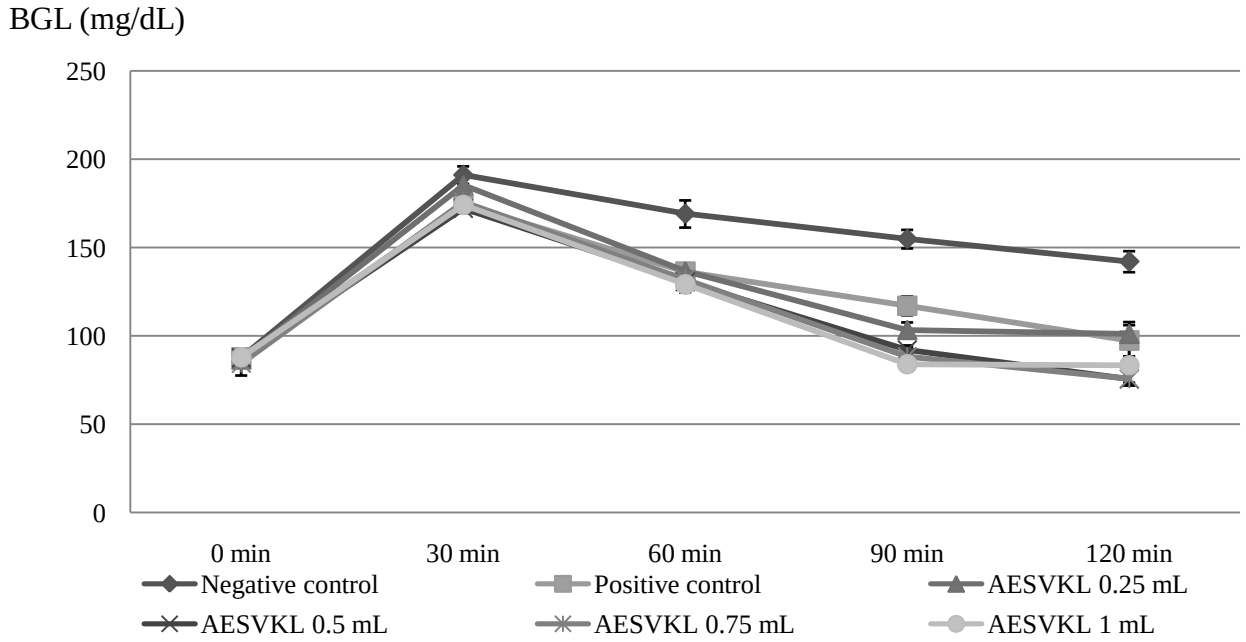


Figure 1. Effect of AESVKL on oral glucose tolerance test (n=4). *P<0.05compared to positive control rats.

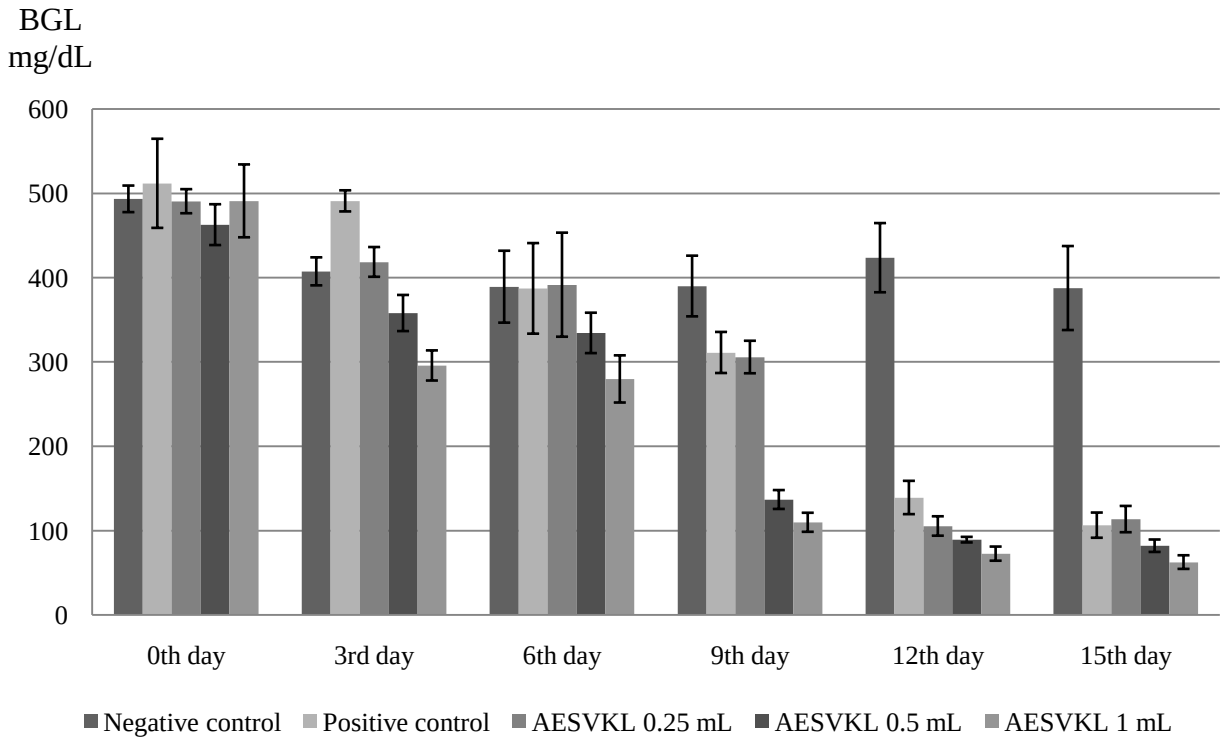


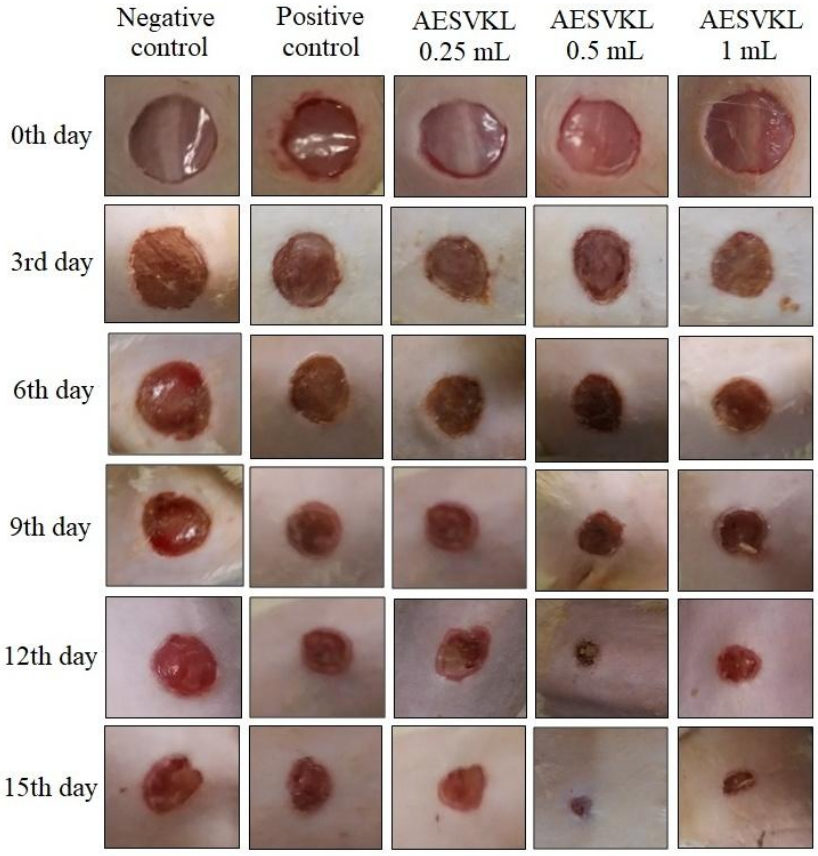
Figure 2. Effect of AESVKL on blood glucose in STZ induced hyperglycemia rats (n=5).

Strepzotosin increase blood glucose level of rats (Figure 2) by visible weight loss within 7-10 days, then this indicates the damage of the irreversible pancreatic langerhans [21]. AESVKL administration for 15 days resulted in a decrease in blood glucose in rats, but in the negative control treatment showed blood glucose levels in the range 387-493 mg/dL. From the test results it can be seen that the decrease in blood glucose levels began to be seen on day 3, the largest decrease in blood glucose levels occurred in AESVKL 1 mL, then AESVKL 0.5 mL and AESVKL 0.25 mL. In this study, AESVKL contained secondary metabolites such as flavonoids, tannins, and saponins, these compounds probably contributed to the decline in blood glucose rats. Flavonoid can reduce blood glucose by stimulating insulin release from pancreatic β-cells that are not damaged, so as to restore pancreatic β-cell function and increase insulin secretion in the body. Flavonoids also reduce glucose absorption, regulate the activity of enzymes involved in carbohydrate metabolism, and inhibit the breakdown of polysaccharides into monosaccharides [22]. Saponins have the potential for antidiabetic activity against insulin secretion due to calcium channel modulation and pancreatic β-cell rejuvenation. Saponins have activity as antioxidants, because they have the power to reduce and eradicate superoxide radicals and metal binding activity [23]. Tannins have astrigent properties that can inhibit the absorption of sugar on the surface of the small intestine so that it can reduce blood sugar levels [24].

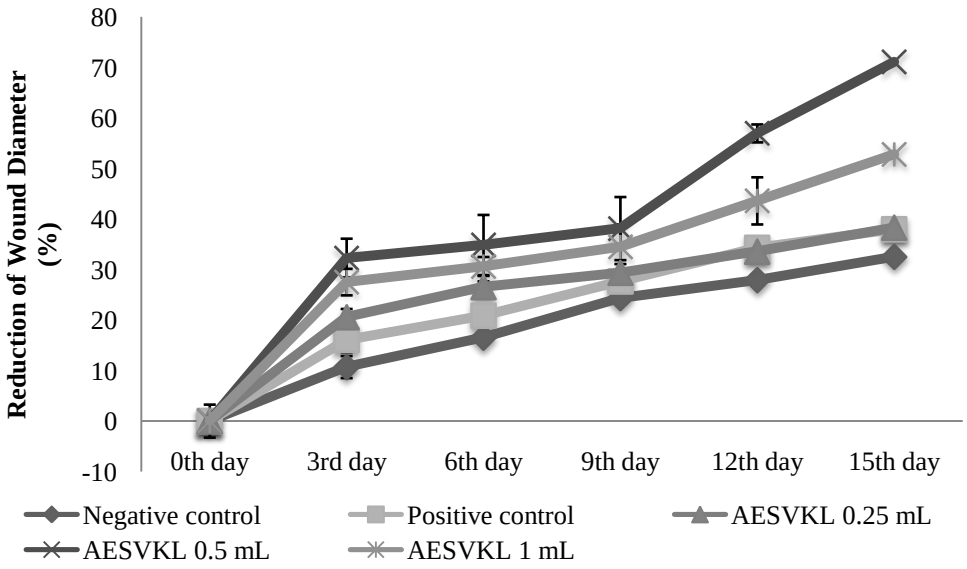
Effect of AESVKL on wound healing

Oral administration of AESVKL (0.25 mL, 0.5 mL and 1 mL) promotes excision wound healing in hyperglycemia rats significantly (($P<0.05$) compared to each other but AESVKL 0.5 and 1 mL promotes excision wound healing in hyperglycemia rats significantly ($P<0.05$) compared to positive and negative control (Figure 3).

Disruption of the integrity of skin, mucosal surfaces or organ tissue results in the formation of a wound. Wounds can occur as part of a disease process or have an accidental or intentional etiology. At the time of insult, multiple cellular and extracellular pathways are activated, in a tightly regulated and coordinated fashion, with the aim of restoring tissue integrity. Classically, this process of wound healing is divided into four distinct phases: haemostasis, inflammation, proliferation and tissue remodelling [25]. After tissue injury, red blood cells and platelets aggregate and form an initial hemostatic plug to protect the wound. Within 24 h, neutrophils enter the wound site and scavenge cellular debris, foreign bodies and bacteria. After 2-3 days, the inflammatory cell population begins to shift to macrophages and fibroblasts appear in the wound site.



(A)



(B)

Figure 3. Effect of AESVKL promotes excision wound healing in hyperglycemia rats (n=5). P<0.05 compared to control rats: photographs of macroscopic appearances of wound excised (A) and reduction of wound diameter (B).

After 3-5 days, the fibroblasts become activated and begin synthesizing collagen. As the collagenous matrix forms, densely packed fibers fill the wound site and during remodeling, reepithelialization of wounds begins within hours of injury and proceeds first over the margin of the residual dermis and subsequently over granulation tissue. The wound gradually becomes stronger with time. However, hyperglycemic conditions in diabetic ulcer slow down this healing process, the healing process is prevented by several abnormalities including prolonged inflammation, impaired neovascularization, decreased collagen synthesis, and defective macrophage functions. [26-27].

The results of this study indicate that oral administration of AESVKL can speed up the healing process of hyperglycemia wounds. Macroscopic findings indicate the reduction in the wound size in the 3rd day to 15th day compared to negative control and positive control. The macroscopic findings indicate its positive effects on the process of wound healing. Literature studies show that plants proven effective at accelerating wound healing have antimicrobial, antioxidant, and anti-inflammatory characteristics [27]. This is related to the phytochemical content of AESVKL, based on the results of phytochemical screening shows that AESVKL has secondary metabolites such as flavonoids, tannins, glycosides, and saponins. AESVKL comprise antioxidants components such as flavonoid, saponin and tannins, antimicrobial components such as saponins and tannins. Flavonoid content in herbal plants has been widely proven to accelerate wound healing by improving epithelialization process. Increased epithelialization and granuloma tissue in the wound may occur due to increased production of collagen and angiogenesis in wounds [28-30]. In addition, saponin and tannin content play a role in tissue regeneration in the process of wound healing [31] and has the ability as a cleanser or antiseptic [32]. The mechanism of action of saponins in wound healing is to stimulate collagen formation which plays an important role in the process of wound closure and increase epithelialization of tissues [33]. Saponins can also increase antimicrobial activity, antioxidants and accelerate epithelial cell migration. Tannins act as antioxidants, antimicrobials, and have hemodynamic effects with vasoconstriction and the making of mechanical blockages to stop mild bleeding [34]. The tannin content accelerates wound healing with several cellular mechanisms that cleanse free radicals and reactive oxygen, increases wound bonding and increases capillary blood vessel formation as well as fibroblasts [35].

Conclusion

Findings of the present study provide a baseline data on excision wound healing and potential of *Saurauia vulcani,* Korth. Leaves to reduce glucose plasma in hyperglycemia rats and supports their traditional claim.

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