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Effect of *Saurauia vulcani* Korth. Leaves on Superoxide Dismutase, HbA1c Levels and Insulin Expression in Hyperglycemic Rats

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Abstract

AIM: This study aimed to investigate *Saurauia vulcani* Korth. leaves. the activity of ethanol extract in hypoglycemic, superoxide dismutase (SOD), glycosylated haemoglobin (HbA1c) and detection of insulin expression by immunochemistry

METHODS: *Saurauia vulcani* Korth. Leaves powder was extracted by maceration method with ethanol 96%. The extract was administrated orally in doses of 100 mg/kg BW for 27 days. Diabetes was induced in rats by administered of Nicotinamide (NA) 230 mg/kg BW and streptozotocin (STZ) 65 mg/kg BW. Level of blood glucose, SOD, HbA1c were measured, and histopathology pancreas was observed to determine insulin expression with immunochemistry

RESULTS: Ethanol extract of *Saurauia vulcani* Korth. Leaves (EESL) shown a significantly (*p < 0.05) reduced in blood glucose levels at 104.25 ± 2.562 mg/dL and HbA1c level at 32.53 ± 0.188 ng/mL, but increased SOD level at 60.64 ± 0.740 pg/mL and histopathology study shown secretion insulin as seen number of expressions of insulin / slice 31.00 ± 0.315.

CONCLUSION: The result of this study showed EESL possess the hypoglycemic activity and increase the level of SOD but decrease the level of HbA1c in diabetic rat condition. The mechanism of the activity is suggested by stimulating the insulin secretion of pancreas β-cells which were damaged.

Introduction

Based on the International Diabetes Federation (IDF) in 2017, shows that the number of diabetic populations in Indonesia reaches 10 million, this number continues to increase every year [1]. The hyperglycemic condition leads to microvascular and macrovascular complications and early death [2]. Diabetes is a state of increased free radical production that elicits oxidative stress as a consequence of an imbalance between radical-generating and radical-scavenging systems [3]. The uncontrolled production of oxygen free radicals and the unrateable system of antioxidant capability in protection results in the cause of many diseases, such as cancer, diabetes, heart diseases, Alzheimer's, and ageing [23]. Higher levels of HbA1c cause complications. According to the American Diabetes Association (ADA) the target value of HbA1c levels in adult DM patients is < 7.0% as a sign of good metabolic control status, general guidelines for reducing the risk of microvascular complications (nephropathy, neuropathy, retinopathy) and macrovascular (coronary heart disease, cerebrovascular disease, and peripheral vascular disease) [4].

Saurauia vulcani Korth. is one of the plants used as antidiabetic traditionally in Tapanuli Utara, North Sumatera, Indonesia. Ethanolic extract of *Saurauia vulcani* Korth. Leaves can reduce blood glucose level in mice which induced by glucose 50% and alloxan at dose 200 mg/kg BW [5]. The purpose of this study was to determine hypoglycemia, HbA1c and insulin expression activities of ethanol extract of *Saurauia vulcani* Korth.

Material and Methods

Plant and chemicals material

The materials used in this study were *Saurauia vulcani* Korth. Leaves from Sipangan Bolon, North Sumatera, Indonesia. The chemicals used are pro-analysis grade: ABTS (Sigma), potassium persulfate (Merck), nicotinamide (NA), streptozotocin (STZ) (Nacalai), sodium CMC (Merck), SOD ELISA kit (FineTest), HbA1c ELISA kit (FineTest), the technical grade of ethanol and distilled water.

Preparation of extract

The air-dried and powdered leaves of *Saurauia vulcani* Korth. Leaves (1 kg) were extracted by cold maceration with ethanol 96% at room temperature on a shake. The filtrate was collected and then evaporated under reduced pressure to give a viscous extract and then freeze-dried to give a dried extract [6].

Preparation of Extract Suspension and NA-STZ Solution

Suspension of the extract was prepared by using 0.5% CMC-Na with a certain concentration. The solution of STZ was prepared by dissolving STZ in distilled water. NA was prepared by dissolving NA in NaCl 0.9%.

Preparation of NA & STZ Induced Diabetic Rat

The rats were induced with NA solution of` 230 mg/kg and STZ solution 65 mg/kg intraperitoneal (IP). The blood glucose level (BGL) of the rat was measured on the 5th day. On the 5th day, rats had BGL higher than 200 mg/dl were separated and used as test animals. Animals with BGL lower than 200 mg/dL, were induced back with NA-STZ. If on the 5th day the BGL of the rat was higher than 200 mg/dL, the animal was ready to be tested.

Study of the antidiabetic effect of ethanol extract of *Saurauia vulcani* Korth. Leaves (EESL) were conducted using NA and STZ induced diabetic rats by a single dose of ethanol extract. Rats were divided into 4 groups and each group consisting of 4 rats, they were: Group I) Diabetes rats were given suspension of 0.5% CMC, dose 1% of body weight (BW); Group II) Diabetic rats were given suspension of EESL with dose 100 mg/kg BW; Group III) Diabetic rats were given suspension of Glibenklamid® with dose 0.45 mg/kg BW, and Group IV) Normal rats (without treatment).

Suspension of tested material (ethanol extract) was administered everyday orally, and the BGL of the rat were measured on the 4th, 8th, 12th, 16th, 20th, 24th and 28th days after administration of the test material [7].

Analysis of SOD and HbA1c by ELISA

To investigate the effect of EESL on the level of SOD and HbA1c in plasma was examined with ELISA. 0.1 mL of plasma was added to the plate, and the procedure was followed based on SOD and HbA1c ELISA kit instruction (FineTest).

Analysis of Insulin by Immunohistochemistry

The reading of immunohistochemical preparations using a light microscope with an automatic camera (Matsuoka Nissei, Japan) at 400 x magnification. The area coloured with anti-insulin antibodies (beta area cell) found to be brown. Data analysis used image raster.

Statistical analysis

All data were analysed with descriptive and ANOVA using SPSS 22.

Results

Antidiabetic Activity

As a result, blood glucose levels were decreased on the 5th day in EESL group at a dose of 100 mg/kg BW. Effect of treatments EESL on blood glucose level in NA & STZ induced diabetic rat were shown in Figure 1.

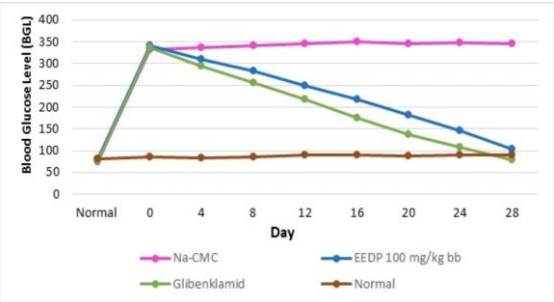

Figure 1: Antidiabetic activity of EESL

The effect of Saurauia vulcani K. on SOD Level

SOD level in plasma was shown to the difference among all the tested groups significantly. The best group (glibenclamide 0.45 mg/kg BW) was

the highest of SOD compared with other groups. There are significant differences between groups (*p < 0.05) which shown in Table 1.

Table 1: SOD plasma level with various treatment

Treatment	SOD level (pg/mL)
Sodium CMC 0.5%	41.30 ± 0.28^{bc}
EESL 100 mg/Kg BW	60.64 ± 0.74^{ac}
Glibenklamid	67.01 ± 0.93^{a}
Normal	68.17 ± 1.26^{a}

The values are expressed as mean ± SEM, n = 4 animals in each group. Statistical analysis to compare the group with another group was done by ANOVA, followed by Tukey test. Values ^{a} was statistically different compared to the CMC group, values ^{b} was statistically different compared to glibenclamide group, values ^{c} was significantly different to the normal group, values *was significantly different p < 0.05.

The effect of Saurauia vulcani K. on HbA1c Level

The nonenzymatic glycation of hemoglobin produces HbA1c. It is an objective marker of average glycemic control in the monitoring of patients with diabetes [19]. HbA1c is also associated with macrovascular outcomes and mortality [20], [21], [22]. The result of HbA1c measurements in this study showed that extract had ability in reducing HbA1c level significantly in the diabetic rat, as seen in Table 2.

Table 2: HbA1c plasma level with various treatment

Treatment	HbA1c level (ng/mL)
Sodium CMC 0.5%	68.52 ± 1.30^{bc}
EESL 100 mg/kg BW	32.53 ± 0.18^{abc}
Glibenklamid 0.45 mg/kg BW	26.08 ± 0.94^{a}
Normal	25.73 ± 1.37^{a}

The values are expressed as mean ± SEM, n = 4 animals in each group. Statistical analysis to compare the group with another group was done by ANOVA, followed by Tukey test. Values ^{a} was statistically different compared to the CMC group, values ^{b} was statistically different compared to glibenclamide group, values ^{c} was significantly different to the normal group, values *was significantly different p < 0.05.

Expression of Insulin

Assessment of pancreatic tissue slices stained with antibodies to insulin was done by calculating the expression of pancreatic beta Langerhans cells that were immunoreactive to insulin (brown) of the 200 cells counted. The average number of expression insulin in Langerhans Island shown in Table 3.

Table 3: The average number of expression insulin

Treatment	Number of expression insulin
Sodium CMC 0.5%	7.55 ± 0.06^{bc}
EESL 100 mg/kg BW	31.00 ± 0.31^{abc}
Glibenklamid 0.45 mg/kg BW	66.62 ± 1.97^{ac}
Normal	85.00 ± 1.25^{ab}

The values are expressed as mean ± SEM, n = 4 animals in each group. Statistical analysis to compare the group with other group was done by ANOVA, followed by Tukey test. Values ^{a} was statistically different compared to the CMC group, values ^{b} was statistically different compared to glibenclamide group, values ^{c} was significantly different to the normal group, values *was significantly different p < 0.05.

In Table 3, glibenclamide showed cell secretion pancreatic beta cells is higher than extract. As seen in Figure 2, it means an increase in expression insulin observed in the group of EESL was not supposed to be caused by the regeneration of β-cells of the pancreas. This increase could be due to an increase in the ability of healthy β-cells to secrete insulin.

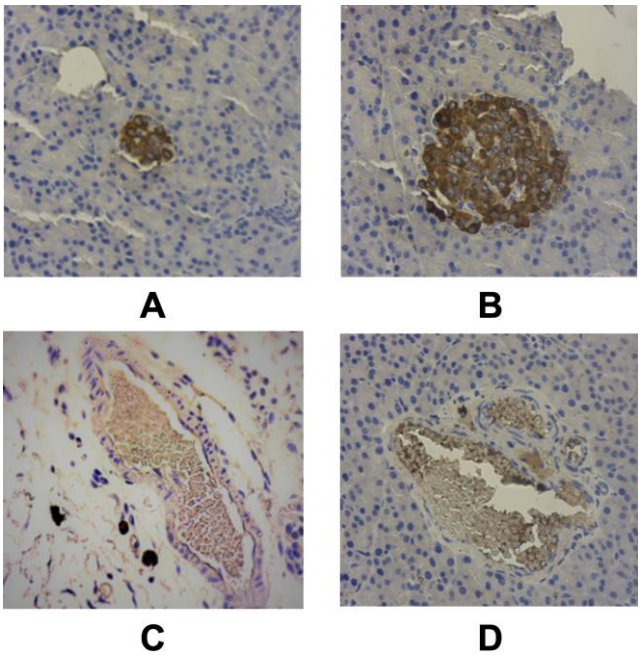

Figure 2: Photomicrograph of the island of Langerhans of each group, 40 × 10; A) Na-CMC; B) EESL; C) glibenclamide; D) normal

Discussion

Streptozotocin has been shown to cause damages directly on pancreatic β cells. STZ cytotoxicity causes the release of free radicals that trigger intracellular oxidative stress. STZ penetrates Langerhans β cells through GLUT 2 glucose transporters. The action of intracellular STZ results in pancreatic DNA β cell changes [8]. DNA damage will activate poly adenosine diphosphate (ADP) ribosylation. Result in the cellular efflux of

nicotinamide adenine dinucleotide (NAD+), and a further reduction in adenosine triphosphate (ATP) which eventually inhibits insulin secretion and synthesis. Nicotinamide which is a precursor of from NAD+ and as a ribose ADP poly inhibitor, will inhibit excessive DNA fragmentation damage causes hepatotoxic to become type 2 diabetes mellitus [9].

A preliminary phytochemical analysis of the EESL was shown flavonoids, steroids/triterpenoids, tannins, glycosides and saponins. Flavonoids, their glycosides and saponins are responsible for blood glucose decreasing activity through increased insulin secretion, as shown in an experiment by NA-STZ induced diabetic rats, which is capable of stimulating pancreatic secretion [10], [11].

High level of SOD caused by the decrease in blood glucose levels. The increase in activity is due to EESL contained flavonoids, some researchers flavonoids work as antioxidants [12]. Flavonoids contained EESL can inhibit oxidation reactions through radical arrest mechanisms (radical scavenging) [13]. Therefore, compounds as antioxidants contained in EESL are non-enzymatic antioxidants that help to increase enzymatic activity (SOD) in its function to capture oxidant compounds, prevent chain reactions and these components are equally important in inducing antioxidant status body [14]. Tanin acts as a free radical catcher and activates antioxidant enzymes so that it can improve pathological oxidative states in diabetes [15]. Saponins act as antioxidants by capturing superoxide and forming hydroxy peroxide, which prevents biomolecular damage caused by free radicals [16].

Increased glycosylation of several proteins, including haemoglobin, had been observed in uncontrolled or poorly controlled diabetes [17] that leads to the formation of HbA1c. HbA1c was found to be increased in diabetic condition, and the amount of increase is directly proportional to the fasting blood glucose level. In the present study, we observed a marked increase in HbA1c level in NA and STZ induced diabetic animals, which could be due to excessive glycosylation of haemoglobin. The decrease in the level of HbA1c in animals given EESL may be due to the decreased level of blood glucose [18].

In conclusion, the result of this study showed EESL possess the hypoglycemic activity and increase the level of SOD but decrease the level of HbA1c in diabetic rat condition. The mechanism of the activity is suggested by stimulating the insulin secretion of pancreas β-cells which were damaged.

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