The effect of *Saurauia vulcani* Korth. leaves extract on penile corpus cavernosa microstructure and the quality of sperm in alloxan-induced diabetic mice

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**Abstract.** The effect of *Saurauia vulcani* K. (pirdot) leaves extract on blood sugar levels, penile corpora cavernous microstructure, and quality of sperm in alloxan-induced diabetic mice have been carried out. Twenty-five healthy and mature male mice of 12 weeks old were divided into five groups in completely randomized design with 5 treatments and 5 replications. The treatments consist of control, alloxan group (alloxan injection: 150 mg/kg bw), three levels of extract groups (alloxan injection plus 150, 200, or 250 mg/kg bw of *S. vulcani* leaves extract) for 8 weeks. The results showed, alloxan treatment increased blood glucose to the level of diabetes, but treatment with *S. vulcani* leaves extracts reduced the glucose to normal. In alloxan-induced diabetic mice, area of corpus cavernosa decreased significantly (p <0.05), and again *S. vulcani* leaves extract decreased corpus cavernosa area to normal. The diameter of helicine artery follows the same trend. In sperm parameter assessment, sperm count in alloxan-induced diabetic mice decreased to 197 x 10⁵, and in extract groups the sperm count were back to normal only in the highest dosage (338 x 10⁵). There were no significant different in sperm quality (motility, viability, and normal percentage). In general, the result confirmed that the antihyperglycemic effect of *S. vulcani* leaves extract, recommended its potency in penile contractile tissue improvement in diabetes, nevertheless it seems to have no effect on sperm quality.

1. **Introduction**

Diabetes mellitus (DM) is a metabolic disease characterized by high blood sugar levels caused by impaired insulin secretion, its action, or both. Predicted global diabetes prevalence was 2.3% in 2000 and 4.4% by 2030, with the number of patients estimated to increase from 171 million to 368 million people [1]. Chronic diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, blood vessels, and male reproductive function [2]. Diabetes mellitus affected male reproduction by interfering with spermatogenesis and penile erectile function [3]. Therapeutic steps for the treatment of diabetes are the use of insulin and other drugs, such as sulfonylurea analogs, alpha-glucosidase inhibitors, and biguanides. Those drugs have certain side effects, such as causing hypoglycemia at higher doses, liver organ problems, lactic acidosis, and diarrhea [4].

In North Sumatra, Indonesia, the use of plant-based ingredients for diabetes therapy has long been recognized. One species with that potential is *Saurauia vulcani* Korth (pirdot). Previous studies have reported *S. vulcani* extract has an antihyperglycemic effect equivalent to the effect of metformin,
a golden standard drug for diabetes [5] [6]. Although some studies have shown that S. vulcani leaves extract is able to decrease blood glucose level, but so far no information has been obtained about its effect on male reproductive function, including the effect on the contractile structure of the corpus cavernosa, and the quality of spermatozoa in diabetic conditions.

2. Materials and Methods

2.1 Plant Material and Extraction

The leaves of S. vulcani were collected from Aek Nauli Forest, District of Simalungun, North Sumatra. For making extracts, leaves are dried, then blend. A total of 100 grams of powder was extracted using a maceration method with methanol solvent. The extract was stored in the refrigerator at 4ºC until it is used further.

2.2 Animal Experiments

The experimental animals used were mice (Mus Musculus L.), male, healthy, age ± 3 months, weighing 25-30 grams. Animals were obtained from and maintained in animal cages Dept. of Biology FMIPA USU Medan. Mice are kept in a special cage for experiments, fed pellets and drinking water (tap water) were accessed ad libitum. Maintenance and treatment of mice in accordance with the recommendations of ethical research approval. Induction of diabetes in mice was done by a single intramuscular injection of alloxan in the thigh with a dose of 150 mg/kg bw. Blood glucose level (BGL) was checked four days after alloxan injection, all mice with BGL > 200 mg/dL was considered diabetes and subsequently included in the experiment.

2.3 Experimental Design

The experiment was arranged in a completely randomized design (CRD). Twenty-five diabetic mice (BGL> 200) were divided randomly into 5 treatment groups. The treatment groups were: K0 (normal), KA (alloxan control), and three groups of extract (P1, P2, and P3), received extract at a dose of 150, 200, and 250 mg/kg bw respectively. The extract was daily administered orally by gavage for 30 days.

2.4 Blood Glucose Levels

Blood glucose level was measured in blood taken from the end tail of the mouse using an Autocheck® glucometer. Blood glucose level was measured weekly for eight weeks.

2.5 Testis Weight

Animals were anesthetized with ether and sacrificed with a cervical dislocation technique while in an unconscious condition. The animal testes were isolated and weighed using a digital scale that has an accuracy of 0.01 g.

2.6 Sperm Characteristic Evaluation

Semen was isolated from the cauda epididymis immediately after testis isolation. Suspension of spermatozoa was made in 0.9% sodium chloride solution. Sperm quantity and sperm quality characteristic were evaluated as previously described [7].

2.7 Preparation of Tissue Section

The preparation of the penis and testis sections was made by paraffin method. All section were stain with Haematoxylin and Eosin. In the male reproductive organ, we observed the microstructure of corpus cavernosa and helicin artery. Measurement of corpus cavernosa area and the diameter of helicin artery was done with the help of Axio Vision 4.8 computer program (Carl Zeiss Inc.). By
drawing a line around the structure with this software, we obtained the data of the total area, diameter, or thickness of the structure automatically. We performed the same technique to measure the diameter of seminiferous tubules in the testis section.

2.8 Data analysis

Data were analyzed statistically by using ANOVA. Post hoc analysis (Duncan’s Multiple Range Test) was applied to all significant results.

3. Result and Discussion

3.1 Blood Glucose Levels

The result showed blood glucose level (BGL) on the control group (K0) was 100 mg/dL up to slightly above it, which was in the range of normal (normal value <120 mg/dL). In the alloxan group (KA), BGL increase sharply after alloxan injection, and a high level (above 300 mg/dL) tended to persist until the end of the experiment. The increase in BGL due to alloxan injection has been widely reported and is the reason for its use as a diabetes-inducing agent in experimental animals. A comprehensive review suggests that alloxan selectively destroys pancreatic beta cells without disrupting other body cells. This is due to the sensitivity of highly pancreatic beta cells to the effects of alloxan free radicals [8]. Alloxan and its metabolite dialuric acid molecules formed superoxide radicals which subsequently undergo dismutation to H2O2 and by the reaction of Fenton formed a highly reactive hydroxyl radical. Destruction of beta cells occurs as a result of a simultaneous ROS action with a massive increase of calcium concentration in cytosolic of beta cells.

Figure 1. The effect of Saurauia vulcani K. leaves extract on blood glucose level in alloxan-induced diabetes mellitus. KO: control (normal), KA: diabetic group (alloxan injection, 150 mg / kg bw), P1, P2, P3 (extract group; diabetic mice plus S. vulcani extract at dose of 150, 200, or 250 mg / kg bw respectively.

In extract groups, the BGL level also increased after alloxan injection, but it decreased after administration of the extract (Figure 1). The effect of the extract began to be noticeable in the third week and continued until at the end of the experiment. At the end of the experiment, the BGL level of all doses tested had restored to a level which was not significantly different from the control (p> 0.05). These results indicate that S.vulcani leaf extract ables to repair the adverse effects of alloxan, as indicated by the decrease in BGL. Previous results showed the same trend5,6. The antidiabetic effect of leaf extract from the same genus (S. roxburghii) has been reported associated with highly extreme antioxidant properties in the DPPH test and is suspected to be related to the phenolic (flavonoid)
component content of the extract [9]. Nevertheless, a comprehensive conclusion that S. vulcani extractable to increase the population of pancreatic beta cells cannot be summarized from this result. It still required histological data and molecular regeneration of pancreatic cells.

### 3.2 Testis Weight and Diameter of Seminiferous Tubules

The results showed the weight of the testes in the control group was 0.14 g. In the alloxan group, the testicular weight was only 0.09 g (Table 1). It seems that alloxan affected testes negatively. It is usual in a study if a treatment affected an organ negatively, then the manifestation that appears will be marked on the growth of the organs. In this experiment, the condition of diabetes triggered by alloxan seems to affect the testes. This is a further effect of diabetes on the reproductive system with the potential causes: impaired HPG-axis function, increased DNA damage, oxidative stress, modulation of cellular pathways, and impair mitochondrial function [10] [11].

| Dose (mg/kgd) | Testis Weight (g) | Seminiferous tubes diameter (μm) |
|--------------|-------------------|---------------------------------|
| 0 (Control)  | 0.14±0.00548a     | 571.04±78.91 a                  |
| Alloxan      | 0.09±0.01095b     | 371.06±138.38b                  |
| 150          | 0.14±0.01140a     | 444.56±133.75 ab                |
| 200          | 0.14±0.00837a     | 301.08±31.89b                   |
| 250          | 0.14±0.01304a     | 315.84±107.38b                  |

In the extract groups, the weight of the testes restored to the normal was demonstrated by statistical analysis which did not differ significantly between the extract groups and control (p> 0.05). The same trend is also found in the diameter of seminiferous tubules. In the control group, the average diameter of the tubule is 571 μm, which in alloxan group decreased to 371. This means that the data of the testis decreasing is in line with the data of seminiferous tubule diameter. It appears that the alloxan or the diabetic condition it triggers causes a serious structural impairment of the testes and the tubules in it. In the extract groups, the leaves extract of S. vulcani for up to 8 weeks of administration did not seem to have fully restoring the condition of seminiferous tubules (Table 1). The trend of improvement is apparent, but until the end of the experiment, normal conditions have not been achieved. The recovery effect can be explained as a consequence of BGL decrease. Their improvement seems to relief HPG-axis stress, a pathway in the endocrine system whose response to hormone secretion can progress immediately depending on the presence of a stress factor [12].

### 3.3 Corpus cavernosa microstructure

The observations made on the corpus cavernosa microstructure include the area of the corpus cavernosa and the diameter of the helicine artery. Both act as the structural part of the penis that determines its erection, because they contain most of the blood in the penis during an erection. The measurements of the corpus cavernosa showed the area of the sponge-like regions of the erectile tissue was 1453 μm². The decrease of the corpus cavernosa area might be related to the reduction of their cellular density (endothelial and smooth muscle) or extracellular matrix component. In rabbit, there is a decrease of collagen component up to 45% which affects blood flow and tissue resistance, and may be the basis of erectile disorders [13]. In alloxan group, the area decreased to 838 μm². It seemed that S. vulcani extract treatment restored the condition to normal, where the diameter at all three doses did not differ significantly with the diameter of control (p> 0.05). The same trend was obtained in the data of lumen diameter of the helicine artery (Table 2).
Table 2. Effect of methanol extract of the *S. vulcani* leaves on micrometry of penis

| Dose (mg/kgd) | Area of corpus cavernosa (μm²) | Lumen diameter of helicine artery (μm) |
|---------------|--------------------------------|--------------------------------------|
| 0 (Control)   | 1453.59 ± 239.94a              | 63.19 ± 34.64ab                      |
| Alloxan       | 838.32 ± 432.41b               | 31.43 ± 9.93a                        |
| 150           | 1441.19 ± 243.30a              | 60.74 ± 21.62ab                      |
| 200           | 1270.63 ± 288.25a              | 67.09 ± 28.5ab                       |
| 250           | 1423.38 ± 289.87a              | 75.86 ± 23.84b                       |

3.4 Quality Characteristic of Sperm

The results on epididymal sperm parameters are presented in Table 3. From all of the sperm parameters observed significant effects only found on sperm counts, and the results were not conclusive. The control group's sperm count is 254 x 10⁵, while in the alloxan group is 197 x 10⁵. In extract groups, it appears that only the highest dose is capable of restoring sperm counts to normal conditions. Statistically, sperm motility, viability, and normality (head) did not show significantly different results.

Table 3. Effect of methanol extract of the *Saurauia vulcani* leaves on semen characteristic

| Dose (mg/kgd) | Sperm Count x 10⁵ sel | Sperm Motility (%) | Viable Sperm (%) | Normal Sperm (%) |
|---------------|----------------------|--------------------|------------------|------------------|
| 0 (Control)   | 254.0±99ab           | 80.9±18.6          | 90.3±9.1         | 97.0±2.6         |
| Alloxan       | 197.4±64a            | 51.5±7.6           | 78.4±12.5        | 88.8±15.2        |
| 150           | 188.6±75a            | 53.3±27.7          | 67.7±20.9        | 72.7±29.6        |
| 200           | 157.8±55a            | 61.5±22.8          | 76.9±17.1        | 86.5±14.8        |
| 250           | 338.6±84b            | 50.2±15.4          | 81.3±8.2         | 89.5±5.5         |

In general, our result confirmed the antihyperglycemic effect of *S. vulcani* leaves extract, recommended its potency in penile contractile tissue improvement in diabetes, but seems to have no effect on sperm quality.

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