Antihyperglycaemic and haematological effects of ethanol extract of *Salacia lehmbachii* leaf in alloxan-induced diabetic Wistar rats

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**ABSTRACT**

**Introduction:** *Salacia lehmbachii* is used traditionally for the treatment of diabetes mellitus. This study investigated the hypoglycaemic potentials of ethanol leaf extract of *S. lehmbachii* and its effects on alloxan-induced diabetic rats’ haematological parameters.

**Methods:** A total of 36 male Wistar rats including normal, diabetic untreated and diabetic treated ones were used in this study. Diabetes was induced by a single intraperitoneal injection of 150 mg/kg of alloxan. When confirmed diabetes, the rats were orally administered 100 mg/kg, 200 mg/kg, and 400 mg/kg daily of the leaf extract for 21 days. The blood glucose levels and haematological parameters were determined in diabetic treated rats compared with the controls.

**Results:** The ethanol leaf extract of *S. lehmbachii* significantly reduced the blood glucose levels of alloxan induced diabetic rats when compared with the diabetic control (*P* < 0.01). The extract also significantly (*P* < 0.01) improved the haematological parameters of treated rats at three doses employed.

**Conclusion:** It can be concluded that the ethanol leaf extract of *S. lehmbachii* possesses antihyperglycaemic properties and could be considered a potential candidate for the development of new drugs in the treatment of anemic conditions.

**Implication for health policy/practice/research/medical education:** In this study, *Salacia lehmbachii* ethanol leaf extract significantly demonstrated antihyperglycaemic activity and improved haematological parameters. Hence, this plant can be considered as a candidate for bioassay-guided and isolation of compounds, which could possibly be developed into new lead structures for drug development programs against diseases. Please cite this paper as: Akuodor GC, Udia PM, Ohadoma SC, Obi IM, Megwas AU, Chilaka JU, et al. Antihyperglycaemic and haematological effects of ethanol extract of *Salacia lehmbachii* leaf in alloxan-induced diabetic Wistar rats. J Herbmed Pharmacol. 2021;10(4):394-400. doi: 10.34172/jhp.2021.46.

**Introduction**

Diabetes mellitus has been considered as one of the present major health concerns globally. The alarming rate at which diabetes is increasing daily with a rapid increase in mortality burden shows that this disease is a leading cause of death worldwide (1). It is the most common endocrine disorder, and has been affirmed that over 300 million people are likely to be diabetic by 2030 (2). This is because managing the disease with synthetic antidiabetic agents without side effects is still a medical challenge (3,4). Despite numerous available antihyperglycaemic agents, herbal remedies are used with success to treat this disease (5). However, attention is presently being focused on herbal drugs for the treatment of diabetes throughout the world. Most of these medicinal plants are used in the treatment of diabetes and its associated complications. Furthermore, based on the WHO recommendations, investigations on antihyperglycaemic agents from medicinal
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Plants have become more important (6,7). More so, these plants’ antihyperglycemic properties are probably due to their ability to restore the function of pancreatic tissues resulting from an increase in insulin output or decreased intestinal absorption of glucose (8). Thus, these herbal agents have \( \beta \)-cells protecting activities and smoothing out fluctuation in plasma glucose levels (9,10).

\textit{Salacia lehmbachii} Loes is a shrub-like to small tree of about three meters high belonging to the family Celastraceae. The leaves are seasonally evergreen, firm, and difficult to slice. The plant is richly found in the tropical rain forest of Central, west, and East Africa (11). There are different therapeutic applications of \textit{S. lehmbachii}, which actually justify its folkloric background. Previous studies have reported the antipyretic, anti-diarrhoea, antimotility, and anti-ulcer of this plant leaf extract (12,13). The root bark extract has been shown to possess analgesic, anti-inflammatory, anticholinergic, anti-infertility activities (14-16). The mixture of the root has also been used traditionally for the treatment of hemorrhoids (17). In addition, the stem bark extract has also been reported to possess antidiabetic and antihyperlipidemic activity (1). The present study aimed at evaluating the antihyperglycemic and haematological effects of the ethanol extracts of \textit{S. lehmbachii} leaves in alloxan-induced diabetic Wistar rats.

Materials and Methods

Plant collection

Fresh leaves of \textit{S. lehmbachii} were collected from farmland in Ukanafun, Nigeria. The plant was identified and authenticated by a taxonomist in the Herbarium Unit of Department of Botany, University of Calabar, Calabar, Nigeria. A voucher sample (herbarium No 688) was prepared and deposited in the Herbarium of Botany Department for reference. The international plant number index is Bot. Jahrb. Syst. 44(2-3):173 (1910).

Leaf extract preparation

The leaf material of \textit{S. lehmbachii} was air-dried to constant weight in the laboratory, powdered, and stored in an airtight container for further use. The dried material was then pulverized using a wooden mortar and pestle. Three hundred and fifty grams (350 g) of the leaf powdered plant material was extracted in 1.5 L of ethanol (75%) with constant shaking for 24 hours. The extract was filtered using Whatman No.1 filter paper. The filtrate was dried on a water bath at a reduced temperature of 40°C and a yield of 18.5 g dry extract with a greenish colour. The extract was later reconstituted in distilled water to give desired doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight.

Phytochemical analysis

The phytochemical screening of ethanol leaf extract of \textit{S. lehmbachii} was carried out for various secondary metabolites such as tannins (ferric chloride test), alkaloids (Mayer’s and Dragendorff reagent test), saponins (Froth test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test), flavonoids (ammonia and sulphuric acid test), glycosides (Keller-Kiliani test), reducing sugars (Fehling’s test), phenol (ferric chloride test) and anthraquinones (Borntrager’s test) (18,19).

Animals

Male Wistar rats weighing between 170 and 190 g were obtained from the animal house of the University of Nigeria, Nsukka. Animals were maintained under standard environmental conditions at 45%–55% relative humidity for 12 hours, each of dark and light cycle and fed with a standard pellet rat diet obtained from Oladokun feed, Ibadan, Nigeria, and water was supplied \textit{ad libitum}.

Acute toxicity test

The LD\(_{50}\) of the plant extract was tested to determine the safety of the agents using OECD (20) method. The study was carried out in two phases, on male Wistar rats. The animals were grouped into three in the first phase, each group having three rats. The rats were administered \textit{S. lehmbachii} leaf extract at doses of 100, 600, and 1000 mg/kg using an orogastric cannula and observed for signs of toxicity and mortality for 24 hours. In the second phase of the study, rats were designated into three groups, with each having one rat and orally administered 2000 mg/kg, 3000 mg/kg, and 5000 mg/kg of the leaf extract. These animals were first observed for 4 hours and later 24 hours. Toxicity signs and mortality were recorded for each group after the observation period. The experimental rats were monitored further for 72 hours.

Induction of diabetes

Overnight fasted rats were made diabetic by a single intraperitoneal injection of freshly prepared alloxan of 150 mg/kg in sterile saline. Seventy-two hours after alloxan injection, animals having blood glucose level >200 mg/dL were considered as being diabetic and recruited for the study (21).

Pharmacological intervention

Rats used for this study were randomly divided into 6 groups with 6 rats each: group 1 rats (normal control) were administered distilled water (10 mL/kg), group 2 rats served as diabetic control, and were also administered distilled water (10 mL/kg). Groups 3, 4, and 5 were diabetic rats treated with \textit{S. lehmbachii} leaf extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. However, group 6 diabetic rats (positive control) were treated with glibenclamide (10 mg/kg). All treatments were carried out through oral cannula for a period of 21 days. Blood glucose levels were measured using Accu-Check glucometer test strips (Roche, Germany) at random on days 0, 7, 14, and 21 of the experiment. Blood samples from the tail vein
puncture for measurement of glucose levels were collected 24 hours after under halothane anaesthesia, while samples for determination of haematological parameters were collected by cardiac puncture from rats and dispensed into EDTA containers and analyzed within 2 hours of collection.

Determination of haematological parameters
Mythic 18 haematological analyzer (Orphee, Switzerland) was used to determine the haematological components, including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs), platelet count (PLC), differential count (neutrophils, lymphocytes, monocytes, eosinophil and basophils), and red cell distribution width (RCDW).

Statistical analysis
Results were expressed as means ± SEM and analyzed with statistical package for social sciences (SPSS version 20) by using one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test. A difference in the mean P<0.05 was considered significant.

Results
Phytochemical analysis
The phytochemical screening of S. lehmbachii ethanol leaf extract contained secondary metabolites such as alkaloid, saponins, tannins, flavonoids, terpenoids, steroids, glycosides, phenol, reducing sugar, while anthraquinone was not detected.

Acute toxicity test
The ethanol leaf extract of S. lehmbachii did not produce any lethality or significant toxicity signs in rats up to 5000 mg/kg bodyweight for 24 and 72 hours post-treatment.

Effect of the ethanol leaf extract of Salacia lehmbachii on blood glucose levels in Wistar rats
Antihyperglycaemic impact of the leaf extract on blood glucose levels of the experimental rats is shown in Table 1. Intraperitoneal alloxan administration into the rats significantly elevated blood glucose levels in Wistar rats compared with control (normal) rats. The blood glucose levels increased from 88.52 to 240.58 g/dL. Treatment with the ethanol leaf extract of S. lehmbachii at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg significantly (P<0.01) reduced the blood glucose levels in dose dependent fashion compared with diabetic untreated rats. However, 400 mg/kg of the extract with the highest activity in the extract-treated groups compared favourably well with the glibenclamide (10 mg/kg) treated group.

Effect of the leaf extract on haematological indices in rats
Table 2 shows a significant (P<0.05) decrease in the levels of RBC, Hb, PCV, MCH, MCV, MCHC, RCDW as well as glibenclamide, after intraperitoneal administration of alloxan when compared with the normal control group. However, following intervention with the leaf extract and glibenclamide at different doses applied, these haematological indices significantly (P<0.01) increased compared with diabetic untreated group.

Effect of Salacia lehmbachii ethanol leaf extract on WBC differential in Wistar rats
There was significant (P<0.05) observed reduction in serum WBC, neutrophils, lymphocyte, monocytes, eosinophils, basophils, and platelets following induction of hyperglycemia in rats with alloxan compared with normal control. The leaf extract significantly (P<0.01) increased the levels of these parameters at both doses used when compared with the diabetic control group (Table 3). The best results were observed at 400 mg/kg of the extract and the standard drug (glibenclamide 10 mg/kg).

Effect of ethanol extract of Salacia lehmbachii leaf on body weight of Wistar rats
There was a significant (P<0.05) weight increase in all the rats given various doses of the extract and those treated with glibenclamide. However, there was a considerable weight loss in diabetic control rats (Table 4).

| Treatment       | Dose (mg/kg) | Blood glucose level (mg/dL) | Day 0 | Day 7 | Day 14 | Day 21 |
|-----------------|-------------|-----------------------------|-------|-------|--------|-------|
| Normal control  | 10 mL/kg    |                             | 88.52 ± 2.22 | 91.45 ± 2.40 | 93.48 ± 2.52 | 74.52 ± 2.34 |
| Diabetic control| 10 mL/kg    |                             | 230.40 ± 2.42 | 240.22 ± 3.25 | 269.68 ± 3.88 | 298.44 ± 4.20 |
| S. lehmbachii   | 100         |                             | 238.39 ± 2.25 | 220.52 ± 2.20* | 175.49 ± 2.10** | 135.45 ± 2.02** |
|                 | 200         |                             | 240.58 ± 2.35 | 190.28 ± 2.30** | 152.77 ± 2.20** | 128.10 ± 2.08** |
|                 | 400         |                             | 235.52 ± 2.32 | 182.55 ± 2.23** | 142.85 ± 2.17** | 118.18 ± 2.02** |
| Glibenclamide   | 10          |                             | 230.49 ± 2.20 | 173.14 ± 2.19** | 135.59 ± 2.14** | 110.06 ± 2.06** |

Values are mean ± SEM (n = 6); * P<0.05 when compared with control group; ** P<0.01 when compared with diabetic control group.
Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelet count (PLC). SL: 100, 200 and 400 = S. lehmbachii in mg/kg, respectively.

Values are mean ± SEM (n=6); *P<0.05 when compared with control group; **P<0.01 when compared with diabetic control group.

Table 4. Effect of ethanol extract of Salacia lehmbachii leaf on body weight of alloxan-induced diabetic Wistar rats

| Treatment          | Dose (mg/kg) | Day 0       | Day 7       | Day 14      | Day 21      |
|--------------------|--------------|-------------|-------------|-------------|-------------|
| Control            | 20 ml/kg     | 185.50 ± 1.44 | 198.65 ± 3.13 | 210.66 ± 3.40 | 215.69 ± 3.29 |
| Diabetic control   | 20 ml/kg     | 189.90 ± 2.67 | 185.30 ± 2.43 | 171.51 ± 1.52 | 164.34 ± 1.40 |
| S. lehmbachii      | 100          | 190.00 ± 1.42 | 197.80 ± 2.43* | 207.60 ± 2.62* | 214.11 ± 2.50* |
|                    | 200          | 188.75 ± 1.24 | 195.38 ± 2.40* | 205.80 ± 2.35* | 210.10 ± 2.06* |
|                    | 400          | 190.30 ± 1.37 | 197.30 ± 1.40* | 205.55 ± 2.36* | 211.48 ± 2.40* |
| Glibenclamide      | 10           | 190.60 ± 2.66 | 198.40 ± 2.33* | 206.78 ± 2.64* | 211.64 ± 2.13* |

Values are mean ± SEM (n=6); *P<0.05 when compared with control group.

Discussion

Plant parts have been used globally in traditional medicine for the treatment of different diseases and disorders. More than two thousand species of medicinal plants have been scientifically recorded as herbal medicines for diabetes. These agents have been widely acknowledged to be safe with minimum side effects, efficacious, and affordable (22-24). This study investigated antihyperglycemic and haematological activities of S. lehmbachii ethanol leaf extract in alloxan-induced hypoglycemic rats for 21 days. Its phytochemical analysis was equally carried out, and the result indicates the presence of a variety of phytoconstituents in the extract. Each of these constituents is known for its various protective and therapeutic effects (25).

Alloxan selectively destroys the insulin-producing beta cells found in the pancreas. Hence it affects endogenous insulin release and, as a result, increases blood glucose level (26). The repeated administration of different doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) of the ethanol extract of S. lehmbachii leaf for 21 days resulted in a significant decrease in blood glucose levels in alloxan-induced hyperglycemic rats.
induced diabetic rats with a higher decrease observed in 400 mg/kg. Medicinal plant extracts have been reported to possess valuable antidiabetic properties and a stimulatory insulin release activity. (27). However, the reference drug (glibenclamide 10 mg/kg) demonstrated higher activity than the leaf extract to reduce the blood glucose level compared with diabetic control. It has been established that glibenclamide produces antidiabetic activity by increasing insulin secretion from the existing pancreatic β-cells (28). In the present study, the observed antihyperglycemic action of S. lehmbachii leaf extract may be by potentiating the insulin effect, probably by increasing the pancreatic secretion of insulin from the β-cells or by modulating the intracellular glucose utilization (29). Thus, the antihyperglycemic effect of S. lehmbachii leaf extract may be linked to the presence flavonoids, terpenoids and other secondary metabolites in the leaf extract. These phytoconstituents have been implicated in the antidiabetic activities of many plants (30,31).

More so, the antihyperglycemic properties of S. lehmbachii leaf is comparable to the stem bark extract (S. lehmbachii), which has demonstrated significant antidiabetic and antihyperlipidemic effect on alloxan-induced diabetes in rats (1) Furthermore, oral administration of the extract significantly improved the weight of diabetic rats probably due to protective effect of the extract on tissue structural constituents (1).

Moreover, the assessment of haematological indices could be used to show the detrimental effect of most plant extracts on blood constituents of animals. These parameters are equally used to ascertain possible alterations in the levels of biomolecules like metabolic products, haematology, normal functioning, and histomorphology of organs (32,33).

The anaemic condition in diabetes mellitus has been due to the increased non-enzymatic glycosylation of red blood cell membrane proteins (32). In the present study, the red blood cell parameters, which include Hb, PCV, MCV, MCH, MCHC, and RCDW were assessed to determine the beneficial effect of S. lehmbachii on the levels of anaemic diabetic rats. However, the study revealed significant depletion of haematological parameters in the hyperglycemic untreated rats compared to the normal control. These abnormalities have been implicated in anaemic condition in man (34,35). Administration of the ethanol extract of S. lehmbachii elicited a positive change in the levels of haematological indices of diabetic rats, especially at 400 mg/kg. Hence, an increase in RBC and its related indices by the leaf extract is an indication of its ameliorative effect on anaemic condition in alloxan-induced diabetic rats.

Furthermore, there was a significant reduction in the levels of WBC, neutrophils, lymphocytes, monocytes, eosinophils, and basophils in alloxan-induced diabetic rats compared to the normal group. The extract at both doses applied markedly improved the levels of WBC, neutrophils lymphocytes, monocytes, eosinophils, and basophils compared with glibenclamide treated group. In addition, there was a decrease in the level of platelet in alloxan-induced diabetic rats compared to normal. The platelets’ ability to cause aggregation in diabetic animals with poor glycaemic control probably resulted from insufficient insulin (29). Prolonged depletion of platelet could lead to internal and external bleeding and may result in death. The continuous administration of the leaf extract of S. lehmbachii showed improvement in the level of the platelets. However, the observed activity of the extract suggests that it can control bleeding. The acute toxicity studies showed the non-toxic nature of S. lehmbachii ethanol leaf extract. There was no lethality or any toxic reactions observed throughout the duration of the toxicity study.

**Conclusion**

The findings from this study show that ethanol leaf extract of S. lehmbachii may possess anihyperglycemic potentials and improve haematological indices in anaemic conditions. Thus, the information from this study may serve as a guide for the discovery of new antihyperglycemic and antianaemic drugs. Further studies are ongoing in our laboratory to isolate and identify various active principle(s) in the leaf extract responsible for the antihyperglycemic and antianaemic activities.

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**Authors’ contributions**

GCA conceived the research idea and designed the work, GCA, SCO and AUM carried out the experiment, PMU wrote the first draft of the manuscript, IMO carried out the literature search, JUC carried out the statistical analysis, while KCC and FVU supervised the study. All authors read and approved the final manuscript for publication.

**Conflict of interests**

The authors declare no conflict of interest.

**Ethical considerations**

The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee, Faculty of Medicine, Ebonyi State University, Abakaliki (EBSU/DRIC/UREC/04/054) as well as the international guidelines on the Use and Handling of Experimental Animals.

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