



Effect of Ethanolic Leaf Extract of *Sarcocephalus latifolius* on Some Biochemical Parameters in Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GIA and MNN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript.

Authors MNN and CDL managed the analyses of the study. Author MNN managed the literature searches and took care of all laboratory animals. All authors read and approved the final manuscript.

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ABSTRACT

This research project was carried out to investigate the biochemical effect of *Sarcocephalus latifolius* leaf extract in alloxan induced diabetic rats. The study was carried out for about 12 months in the department of Biochemistry University of Jos, Jos, Nigeria and Prestige medical Laboratory, Jos, Nigeria. Twenty four rats were divided into six groups of four animals each (3 male groups and 3 female groups). The male and female rats in group 1 and 2 were induced with alloxan monohydrate (as negative controls and diabetic treated animals respectively) while the third groups were used as positive controls and were given distilled water. The phytochemical constituent of *Sarcocephalus latifolius* leaf extract shows the presence of secondary metabolites such as Tannins, Flavonoids and cardiac glycosides. The weight of negative control groups were significantly ($P < 0.05$) reduced when compared to other groups. Both the male and female rats treated with *Sarcocephalus latifolius* leaf extract, showed significant decrease ($P < 0.05$) in blood

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glucose level compared to the untreated rats. Liver biomarkers (ALT, AST and ALP), significantly decreased ($P < 0.05$) in both male and female diabetic rats after treatment with *Sarcocephalus latifolius* leaf extract compared to the untreated rats. *Sarcocephalus latifolius* leaf extract has hypolipidemic effect and thus can be used in the management of diabetes. The result above shows that the ethanolic leaf extract of *Sarcocephalus latifolius* can be used in the management and/or control of diabetes and other related complications.

Keywords: *Sarcocephalus latifolius*; alloxan induced diabetic rats; hypoglycaemia; glucose; lipid profiles; enzyme activity.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder that precipitates disturbances in glucose, lipid and protein homeostasis [1]. Currently, there is a renewed and growing interest in the use of plant - based products as drugs or as 'leads' in the manufacturing of more potent drugs [2]. Several secondary plant metabolites have been shown to modify biological processes, which may reduce the risk of chronic diseases in humans [3], a decoctions of ubulu-inu, Tafashiya or Oya leaves (*Sarcocephalus latifolius*) is used in South Eastern Nigeria in the management of diabetes. Scientific studies also indicated that leaf extract of *Sarcocephalus latifolius* possesses hypoglycaemic effect [4]. Diabetes mellitus is the commonest non-communicable endocrine disease and is considered one of the leading causes of death all over the world.

The plant African peach (*Sarcocephalus latifolius*) is of the family Rubiaceae. It is a multi-stemmed tree or shrub up to 12 m [5] and the leaves are usually green in colour. It occurs widely throughout Africa. Its generic name is derived from the Greek word sarco (fleshy) and cephalus (headed) in reference to the flowers. The specific epithet is derived from the latin word lati (broad) and folius (leaved). A haemaphrodite tree flowering from April-June and fruits ripen from July-September. Earlier reports on the various medicinal uses of this plant have been reported by many traditional medicine practitioners to be effective in the treatment and management of many ailments such as febrile illness, stomach disorder, cough, malaria fever and jaundices. Others include constipation, dysmenorrhoea abscesses, vomiting and threatened abortions. Clinically *S. latifolius* has been shown to paralyse *Trichostongylus columbriformis* larvae in a concentration dependent manner. Distance to health centres, low availability and high costs of modern medicines enhance its patronage.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The plant materials were obtained with the help of Prof. G. I. Adoga from Naraguta, Jos North in Plateau state. The samples were taken to Federal College of Forestry, Jos where it was identified and authenticated by Mr. Joseph Jeffrey Azila of the Department of Forestry Technology. The sample's voucher/specimen number is FHJ283.

2.2 Preparation of Ethanolic Leaf Extract of *Sarcocephalus latifolius*

Fresh leaves of *Sarcocephalus latifolius* were dried for three weeks to a constant weight at room temperature under continuous ventilation and reduced to coarse powder with the aid of a mortar and pestle. 60 g of the plant's powder was weighed and transferred into 250 ml conical flask. This was soaked in absolute ethanol and allowed to stand for 3 days. The content was then shaken on a mechanical shaker for 3 hours and filtered while cold. The filtrate obtained was evaporated to dryness on a water bath. The dry extract was weighed and preserved in a dessicator until when needed. The dry extract was dissolved in distilled water at appropriate concentrations for the various experimental doses using the equation of Tedong et al. [6].

$$V \text{ (ml)} = D \times P/C$$

Where: V = Volume; D = Dose used (mg/kg body weight); P = Body weight (mg); C = Concentration (mg/ml).

2.3 Phytochemical Screening of the Extract

The ethanolic extract was screened for its phytochemical constituents using standard qualitative procedures [7,8].

2.4 Animals and Biochemical Assays

2.4.1 Sources of animals

The study was conducted using twenty four albino wistar rats of mixed sexes (12 males and 12 females). They were obtained from the animal house unit of pharmacology Department University of Jos. Stainless steel cages with wire mesh floor were used for housing the animals, free access to water was also allowed alongside grower mesh (locally prepared by the animal farm department of pharmacology university of Jos). They were fed for 2 months to attain a required weight between 150 – 170 g.

2.4.2 Administration of Alloxan monohydrate

After 2 months, the rats were divided into six groups of four animals each (3 male groups and 3 female groups).

Diabetes was induced into the rats in group 1 and 2 for both males and females (as diabetic controls and diabetic treated animals respectively) by injecting them with alloxan monohydrate intraperitoneally with dosage of 150 mg/kg body weight while the third groups were used as normal controls. Diabetes was confirmed from the fasting blood glucose after 48 hours of induction using on-call plus test strips.

2.4.3 Collection of blood samples

After 21 days of treatment with 400 mg/kg (body weight) leaf extract, the rats were starved for a night before they were sacrificed by cervical dislocation. The blood samples were collected in clean dry centrifuge tubes and allowed to clot for about one hour and spinned at 300 rpm for 10 mins. The serum samples were collected and transferred to small clean bottles. The clear supernatant was used for the estimation of Glucose, lipid profile, Alanine transaminase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP).

2.4.4 Determination of glucose level

The glucose concentration was determined using enzymatic indicator test based on the trinder reaction which is quantified by the formation of a pink quinonimine dye [9,10].

2.4.5 Determination of plasma lipid profile

Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoproteins (HDL) were

determined using Fortress Diagnostic kits while Low Density Lipoprotein (LDL) was calculated using formula from Friedwald et al. [11].

2.4.6 Determination of liver function test (LFT)

Plasma enzymes such as Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) were determined using Fortress Diagnostic kits.

2.5 Stastical Analysis

Data Analysis was done using the graph pad prism computer software. Student t-test and One Way Analysis of Variance (ANOVA) were used for comparison. *P*-value <0.05 was considered significant.

3. RESULTS

3.1 Phytochemical Screening

The phytochemical screening results shows that *Sarcocephalus latifolius* leaf extract tested positive for Tannins, Flavonoids, Carbohydrates and Cardiac glycosides while Saponins, Anthraquinones, Terpenes and steroids tested negative as shown in Table 1.

Table 1. Phytochemical screening results for *Sarcocephalous latifolius* leaf extract

Phytochemical	Results
Alkaloids	-
Saponins	-
Tannins	+
Flavonoids	+
Carbohydrates	+
Cardiac Glycosides	+
Anthraquinones	-
Terpenes and Steroids	-

Key: (+) Present while (-) absent

3.2 Body Weight in Normal and Diabetic Rat

Table 2a and 2b shows the female and male body weight. Diabetic untreated rats significantly lost weight compared to other rats in other groups.

3.3 Blood serum Glucose Level

The negative control rats showed significant difference (*P*<0.05) in the blood serum glucose

(mmol/L) when compared with the group of positive control rats. Following the administration of *Sarcocephalus latifolius* leaf extract, the blood glucose level also showed significantly difference ($P<0.05$) when compared with the positive control rats as shown in Table 3.

3.4 Enzyme Activity

Table 4a and 4b shows respectively the female and male aspartase amino transaminase activity (AST), alanine amino transferase activity (ALT),

alkaline phosphatase activity (ALP) in the blood serum of the positive and negative control groups.

3.5 Lipid Profile

The extract reduces the serum levels of cholesterol, triglycerides, Low density lipoproteins (LDL) - cholesterol and High density lipoproteins (HDL) – cholesterol in the treated animals as compared to the untreated ones as shown in Table 5a and 5b.

Table 2a. Mean body weight for negative control, diabetic + leaf extract and positive control

Group (Females)	Initial weight (g)	Final weight (g)	%weight gain/loss
1. Negative control	164±1.23	155±0.79	-5.49
2. Diabetic + Leaf extract	167.1±1.02	155±0.94	-12.10
3. Positive control	154±0.87	169±1.14	9.74

Values are mean ±S.D for 4 determinations

Table 2b. Mean body weight for negative control, diabetic + leaf extract and positive control

Group (Males)	Initial weight (g)	Final weight (g)	%weight gain/loss
1. Negative control	166±1.18	146±0.93	-12.05
2. Diabetic + Leaf extract	170±1.02	153±0.94	-11.11
3. Positive control	152.25±2.5	163.75±2.99	11.50

Values are mean ±S.D for 4 determinations

Table 3. The effect of *Sarcocephalous latifolius* leaf extract on blood glucose level in female and male albino rats induced with alloxan

Group	Glucose level (Female)	Glucose level (Male)
Negative control	7.2± 0.17	8.75± 0.04
Diabetic + Leaf extract	5.60 ± 0.31 ^b	3.60 ± 0.13 ^a
Positive control	3.50± 0.21 ^a	3.52 ± 0.03 ^a

Values are mean ± S.D, a= indicates significant difference ($P<0.05$) when comparing positive control group and treated group with negative control group, b= indicates no significant difference ($P>0.05$)

Table 4a. The effect of *Sarcocephalous latifolius* leaf extract on liver marker enzymes in alloxan-induced diabetic female rats

Female groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
1. Negative control	32.00 ±1.83	97.00 ± 1.29	143.00 ± 2.45
2. Diabetic + Leaf extract	83.00 ±2.58 ^a	51.00 ±1.18 ^a	118.00 ±0.70 ^b
3. Positive control	102.50 ± 6.87 ^a	73.00 ±7.30 ^a	118.50 ±1.29 ^b

Values are mean ± S.D, a= indicates significant difference ($P<0.05$) when comparing positive control group and treated group with negative control group, b= indicates no significant difference ($P>0.05$)

Table 4b. The effect of *Sarcocephalous latifolius* leaf extract on liver marker enzymes in alloxan-induced diabetic male rats

Male groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Negative control	36.13±2.78	21.00 ± 1.47	21.00 ± 2.48
Diabetic + Leaf extract	61.50 ±5.20 ^a	49.00 ± 2.94 ^a	70.00 ±2.58 ^a
Positive control	15.00 ± 2.12 ^a	17.00 ±1.47 ^b	33.00 ±1.29 ^b

Values are mean ± S.D, a= indicates significant difference ($P<0.05$) when comparing positive control group and treated group with negative control group, b= indicates no significant difference ($P>0.05$)

Table 5a. The effect of *Sarcocephalous latifolius* leaf extract on lipid profiles in alloxan-induced diabetic female rats

Female group	Cholesterol (mmol/l)	Triglycerides (mmol/L)	LDL – C (mmol/L)	HDL – C (mmol/L)
Negative control	1.90 ± 0.25	0.40 ± 0.10	0.60 ± 0.09	1.20 ± 0.03
Diabetic + Leaf extract	3.00 ± 0.48 ^a	1.50 ± 0.11 ^a	0.55 ± 0.04 ^b	1.70 ± 0.20 ^b
Positive control	2.65 ± 0.22 ^a	1.25 ± 0.13 ^a	0.53 ± 0.07 ^a	1.50 ± 0.15 ^a

Values are mean ± S.D, a= indicates significant difference (P<0.05) when comparing positive control group and treated group with negative control group, b= indicates no significant difference (P>0.05)

Table 5b. The effect of *Sarcocephalous latifolius* leaf extract on lipid profiles in alloxan-induced diabetic male rats

Male group	Cholesterol (mmol/l)	Triglycerides (mmol/L)	LDL – C (mmol/L)	HDL – C (mmol/L)
Negative control	3.83 ± 0.03	2.20 ± 0.31	2.00 ± 0.15	0.40 ± 0.03
Diabetic + Leaf extract	2.20 ± 0.18 ^b	1.40 ± 0.02 ^b	0.20 ± 0.04 ^a	1.30 ± 0.03 ^a
Positive control	1.58 ± 0.03 ^a	1.14 ± 0.04 ^a	0.95 ± 0.03 ^a	0.39 ± 0.04 ^a

Values are mean ± S.D, a= indicates significant difference (P<0.05) when comparing positive control group and treated group with negative control group, b= indicates no significant difference (P>0.05)

4. DISCUSSION

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine have been shown to have genuine utility and about 80% of rural population depends on it as primary health care. The phytochemical screening of ethanolic leaf extract of *Sarcocephalous latifolius* indicates the presence of secondary metabolites like tannins, flavonoids and Cardiac glycosides as shown in Table 1. The presence of these phytochemicals in high concentration accounts for the significant hypoglycemic effect of *Sarcocephalous latifolius*. It has been showed that medicinal plants with hypoglycemic and anti-diabetic effect usually contain high concentration of flavonoids and tannins. The diabetic control rats significantly lost weight (P<0.05) when compared to the normal and treated groups. This may be due to loss in muscle adipose tissue protein and fatty acids. Studies have also reported significant weight reduction in untreated diabetic rats. Alloxan induce diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia which shows significant increase in blood sugar level after periods of 21 days of treatment. The result shows that the different concentration of *Sarcocephalous latifolius* extract exhibited a profound reduction (P<0.005) in blood sugar level of the diabetic albino rats. This finding is in line with the results of Rajasekaran et al. [12] who using both leaf and bark extracts demonstrated the hypoglycaemic effects of the plant. A number of other plants have been

reported to have anti-hyperglycemic and insulin stimulator effects.

The extract of *Sarcocephalous latifolius* caused significant decrease (P<0.05) in the level of plasma AST, ALT and ALP values in both the female and male rats as shown in Table 4a and 4b respectively. These indicate that the extract has hepato-protective potentials. This observation is consistent with earlier report on hepatoprotective potentials of the leaf extracts of *V. amygdalinain* mice. The levels of AST, ALT and ALP have been reported to be increased in alloxan-induced diabetic rats. The increase in the activities of AST, ALT and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which gives an indication of the hepatotoxic effect of alloxan. The increase in ALT and AST activity in diabetes is always due to hepatocellular damage which may result to leakages into the bloodstream [13]. The reversal of transaminase activity in *Sarcocephalous latifolius* treated diabetic rats towards near normalcy is evidence of prevention cellular and tissue damage under diabetic condition [12]. Also the increase in ALP activity in diabetes is normally due to an injury on the liver.

Diabetes induce hyperlipidemia due to excess mobilization of fats from adipose tissue to the under-utilization of glucose. Table 5a and 5b, showed that the ethanolic extract of *Sarcocephalous latifolius* significantly increased the serum HDL – Cholesterol levels while total

cholesterol, triglyceride and LDL-Cholesterol decreased significantly ($P < 0.05$) in both the female and male diabetic treated groups compared to the untreated groups. The result clearly indicates that the administration of ethanolic leaf extract of *Sarcocephalus latifolius* produced hypolipidemic effect. This shows that there are many bioactive constituents present in the extract and hence at the present, it is not certain which of them are responsible for the observed effects. Studies have shown that the presence of tannins and flavonoids play an important role in hypolipidemic effect. High levels of triglycerides, LDL-Chol and VLDL-Chol have been associated with heart disease, insulin resistance and diabetic mellitus. Studies have shown that hyperlipidemia is a recognized consequence of diabetes mellitus. In alloxan induced diabetes, the increase in blood glucose level is normally accompanied by an increase in serum Cholesterol, Triglycerides, Low density lipoprotein (LDL) and decrease in high density lipoprotein (HDL) [14] thus excess fatty acid in plasma produce by diabetes promotes the conversion of excess fatty acid into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed in the liver may be discharged into blood in the form of lipoproteins [15].

5. CONCLUSION

The results of this study show that the ethanolic leaf extract of *Sarcocephalus latifolius* have hepatoprotective actions and suggest that flavonoids present in *Sarcocephalus latifolius* leaf extract may have a major role in this action. Also other phenolic compounds are known to reduce hyperlipidemia in diabetes. Hence, *Sarcocephalus latifolius* might be beneficial in the management of diabetes mellitus since it possess hypoglycemic, hypocholesterolemic and hypolipidaemic properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care were followed, as well as specific national laws were applicable. All experiments have been examined and approved by the Departmental Animal Science Ethical

committee on the Use and Care of Experimental Animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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