

1 SOME BIOCHEMICAL PROPERTIES OF *Sarcocephaluslatifolius* LEAF EXTRACT

2 IN ALLOXAN INDUCED DIABETIC RATS

3 ABSTRACT

4 **Aims:** This research project is carried out to investigate if the *sarcocephaluslatifolius* leaf
5 extract possess hypoglycaemic property and thus can be used for the treatment of diabetes
6 and to determine its effect on some biochemical parameters in alloxan induced diabetic rats.

7 **Place and Duration of Study:** The study was carried out for about 12months in the
8 department of Biochemistry University of Jos, Jos, Nigeria and Prestige medical Laboratory,
9 Jos, Nigeria.

10 **Method:**Twenty four rats were divided into six groups of four animals each (3male groups
11 and 3female groups). Diabetes was induced into the rats in group 1 and 2 for the males and
12 also group 1 and 2 for the females (as diabetic controls and diabetic treated animals
13 respectively) by intravenous injection of alloxan while the third groups were used as normal
14 controls. Diabetes was confirmed from the fasting blood glucose after 48 hours of induction
15 using on-call plus test strips. After 21 days of treatment with leaf extract, the rats were
16 starved for a night before they were sacrificed by decapitation. The blood samples were
17 collected in clean dry centrifuge tubes and allowed to clot for about one hour and pinned at
18 300rpm for 10mins. The serum samples were collected and transferred to small clean bottles.
19 The following parameters were analysed on the serum samples: Glucose, lipid profile,
20 Alanine transaminase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP).

21 **Result:**The phytochemical constituent of *Sarcocephaluslatifolius* leaf extract shows the
22 presence of secondary metabolites such as Tannins, Flavonoids and cardiac glycosides. The
23 weight of diabetic untreated rats were significantly ($P<0.05$) reduced when compared to other

groups. Both the male and female rats treated with *Sarcocephaluslatifolius* leaf extract, showed significant decrease ($P<0.05$) in blood 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 treated with *Sarcocephaluslatifolius* leaf extract compared to the untreated rats. *Sarcocephaluslatifolius* leaf extract has hypolipidemic effect and thus can be used in the management of diabetes.

Conclusion: The result above shows that the ethanolic leaf extract of *Sarcocephaluslatifolius* can be used in the management and/or control of diabetes and other related complications.

Keywords: *Sarcocephaluslatifolius*, 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 (Van den Berghe *et al.*, 2006). 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 (Ogbonnia *et al.*, 2008). Several secondary plant metabolites have been shown to modify biological processes, which may reduce the risk of chronic diseases in humans (Ugochukwu *et al.*, 2003) a decoctions of ubulu-inu, Tafashiya or Oya leaves (*sarcocephaluslatifolius*) is used in South Eastern Nigeria in the management of diabetes. Scientific studies also indicated that leaf extract of *sarcocephaluslatifolius* possesses hypoglycaemic effect (Gidado *et al.*, 2005 and 2008). 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 (*sarcocephaluslatifolius*) is of the family Rubiaceae. It is a multi-stemmed tree or shrubs up to 12m 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 *Trichostongyluscolumbriformis* 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 material was collected from Naraguta, Jos North in Plateau state. It was identified and authenticated by Prof. G. I. Adoga of Biochemistry Department, University of Jos, Nigeria.

2.2. Preparation of Ethanolic Leaf Extract of *Sarcocephaluslatifolius*

Fresh leaves of *Sarcocephaluslatifolius* were dried to a constant weight at room temperature under continuous ventilation and reduced to coarse powder with the aid of a mortar and pestle. 60g of the plant's powder was weighed and transferred into 250ml conical flask. This was soaked in absolute ethanol and allowed to stand overnight. The content was then shaken

69 on a mechanical shaker for 3hours and filtered while cold. The filtrate obtained was
70 evaporated to dryness on a water bath. The dry extract was weighed and preserved in a
71 dessicator until when needed.The dry extract was dissolved in distilled water at appropriate
72 concentrations for the various experimental doses.

73 **2.3. Phytochemical Screening of the Extract**

74 The ethanolic extract wasscreened for its phytochemical constituents using standard
75 qualitative procedures (Trease and Evans, 1984; Sofowora, 1986).

76 **2.4. Animals and Biochemical Assays**

77 **2.4.1. Sources of Animals**

78 The study was conducted using twenty four albino wistar rats of mixed sexes (12 males and
79 12 females). They were obtained from the animal house unit of pharmacology Department
80 University of Jos. Stainless steel cages with wire mesh floor were used for housing the
81 animals, free access to water was also allowed alongside grower mesh (locally prepared by
82 the animal farm department of pharmacology university of Jos). They were fed for 2months
83 to attain a required weight between 150 – 170g.

84 **2.4.2. Administration of Alloxan monohydrate**

85 After 2months, the rats were divided into six groups of four animals each (3male groups and
86 3female groups). Diabetes was induce into the rats in group 1 and 2 for both males and
87 females (as diabetic controls and diabetic treated animals respectively) by intravenous
88 injection of alloxan after 24 hours while the third groups were used as normal controls.
89 Diabetes was confirmed from the fasting blood glucose after 48 hours of induction using on-
90 call plus test strips.

91 **2.4.3. Collection of Blood Samples**

92 After 21 days oftreatment with leaf extract, the rats were starved for a night before they were
93 sacrificed by decapitation. The blood samples were collected in clean dry centrifuge tubes
94 and allowed to clot for about one hour and spinned at 300rpm for 10mins. The serum samples
95 were collected and transferred to small clean bottles. The clear supernatant was used for the
96 estimation of Glucose, lipid profile, Alanine transaminase (ALT), Aspartate transferase
97 (AST) and Alkaline phosphatase (ALP).

98 **2.4.4. Determination of Glucose Level**

99 The glucose concentration was determined using Enzymatic indicator test based on the
100 trinder reaction which is quantified by the formation of a pink quinonimine dye.

101 **2.4.5. Determination of Plasma Lipid Profile**

102 Total Cholesterol, Triglycerides and High Density Lipoproteins (HDL) were determined
103 using Standard methods while Low Density Lipoprotein was determined using Standard
104 methods.

105 **2.4.6. Determination of Liver Function Test (LFT)**

106 ALT, AST and ALP were determined using standard methods

107 **2.5. Stastical Analysis**

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

111 **3. RESULTS**

112 3.1. Phytochemical Screening

113 The phytochemical screening results shows that *Sarcocephaluslatifolius* leaf extract tested
 114 positive for Tannins, Flavonoids, Carbohydrates and Cardiac glycosides while Saponins,
 115 Anthraquinones, Terpenes and steroids tested negative as shown in table 3.1 below.

116 **Table 3.1**

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

117 Key:(++) *Present in moderately high concentration*, (+++) *Present in very high concentration*, (-) *absent*

118

119 3.2 BODY WEIGHT IN NORMAL AND DIABETIC RAT

120 **Table 3.2a**

Group	Initial weight	Final weight	%weight gain/loss
(Females)	(g)	(g)	
1.Diabetic control	164±1.23	155±0.79	-5.49
2.Diabetic+Leaf	167.1±1.02	155±0.94	-12.10

3.Normal control 154±0.87 169±1.14 9.74

121 Values are mean ±S.D for 4 determinations

122 **Table 3.2b**

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

123 Values are mean ±S.D

124 3.3 Blood serum Glucose Level

125 The diabetic rats showed significant difference (P<0.05) in the blood serum glucose
 126 (mmol/L) when compared with the group of normal control rats. Following the
 127 administration of *Sarcocephaluslatifolius* leaf extract, the blood glucose level also showed
 128 significantly difference (P<0.05) when compared with the normal control rats as shown in
 129 Table 3.3 below.

130 **Table 3.3**

Group	Glucose Level (Female)	Glucose Level (Male)
Diabetic Control	7.2± 0.17	8.75± 0.04
Diabetic + Leaf extract	5.60 ± 0.31 ^b	3.60 ± 0.13 ^a
Normal Control	3.50± 0.21 ^a	3.52 ± 0.03 ^a

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

3.4 Enzyme Activity

Table 3.4a and 3.4b shows respectively the female and male aspartate amino transaminase activity (AST), alanine amino transferase activity (ALT), alkaline phosphatase activity (ALP) in the blood serum of the normal and diabetic groups of rats.

Table 3.4a

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

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

Table 3.4b

Male Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Diabetic control	36.13 $\pm$ 2.78	21.00 $\pm$ 1.47	21.00 $\pm$ 2.48
Diabetic + Leaf extract	61.50 $\pm$ 5.20 ^a	49.00 $\pm$ 2.94 ^a	70.00 $\pm$ 2.58 ^a
Normal control	15.00 $\pm$ 2.12 ^a	17.00 $\pm$ 1.47 ^b	33.00 $\pm$ 1.29 ^b

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

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 Tables 3.5a and 3.5b.

152 **Table 3.5a**

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

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

155 **Table 3.5b**

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

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

158 **4. DISCUSSION**

159 Medicinal plants constitute an effective source of both traditional and modern medicines,
 160 herbal medicine have been shown to have genuine utility and about 80% of rural population
 161 depends on it as primary health care. The phytochemical screening of ethanolic leaf extract of

162 *Sarcocephaluslatifolius* indicates the presence of secondary metabolites like tannins,
 163 flavonoids and Cardiac glycosides as shown in table 3.1. The presence of
 164 these phytochemicals in high concentration accounts for the significant hypoglycemic effect
 165 of *Sarcocephaluslatifolius*. It has been showed that medicinal plants with hypoglycemic and
 166 anti-diabetic effect usually contain high concentration of flavonoids and tannins. The diabetic
 167 control rats significantly lost weight ($P<0.05$) when compared to the normal and treated
 168 groups. This may be due to loss in muscle adipose tissue protein and fatty acids. Studies have
 169 also reported significant weight reduction in untreated diabetic rats. Alloxan induce diabetes
 170 by damaging the insulin secreting cells of the pancreas leading to hyperglycemia shows
 171 significant decrease in blood sugar level after periods of 21 days of treatment. The result
 172 shows that the different concentration of *Sarcocephaluslatifolius* extract exhibited a profound
 173 reduction ($P<0.005$) in blood sugar level of the diabetic albino rats. A number of other plants
 174 have been reported to have antihyperglycemic and insulin stimulator effects.

175 The extract of *Sarcocephaluslatifolius* caused significant decrease ($P<0.05$) in the level of
 176 plasma AST, ALT and ALP values in both the female and male rats as shown in Table 3.4a
 177 and 3.4b respectively. These indicate that the extract has hepato-protective potentials. This
 178 observation is consistent with earlier report on hepatoprotective potentials of the leaf extracts
 179 of *V. amygdalin* in mice. The levels of AST, ALT and ALP have been reported to be
 180 increased in alloxan-induced diabetic rats. The increase in the activities of AST, ALT and
 181 ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into
 182 the blood stream, which gives an indication of the hepatotoxic effect of alloxan.

183 Diabetes induce hyperlipidemia due to excess mobilization of fats from adipose tissue to the
 184 under utilization of glucose. Table 3.5a and 3.5b above, showed that the ethanolic extract of
 185 *Sarcocephaluslatifolius* significantly reduce ($P<0.05$) total cholesterol, triglyceride, HDL-
 186 Cholesterol and LDL-Cholesterol in 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.

5. CONCLUSION

The results of this study show that the ethanolic extracts of *Sarcocephalus latifolius* leaf 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

Not applicable

ETHICAL APPROVAL

Not applicable

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