

1 **EFFECT OF ETHANOLIC LEAF EXTRACT OF *Sarcocephaluslatifolius* ON SOME**
2 **BIOCHEMICAL PARAMETERS IN ALLOXAN INDUCED DIABETIC RATS**

3 **ABSTRACT**

4 **Aims:** This research project was carried out to investigate the biochemical effect of
5 *sarcocephaluslatifolius* leaf extract in alloxan induced diabetic rats.

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

9 **Method:** Twenty four rats were divided into six groups of four animals each (3 male groups
10 and 3 female groups). The male and female rats in group 1 and 2 were induced with alloxan
11 monohydrate (as negative controls and diabetic treated animals respectively) while the third
12 groups were used as positive controls and were given distilled water.

13 **Result:** The phytochemical constituent of *Sarcocephaluslatifolius* leaf extract shows the
14 presence of secondary metabolites such as Tannins, Flavonoids and cardiac glycosides. The
15 weight of negative control groups were significantly ($P < 0.05$) reduced when compared to
16 other groups. Both the male and female rats treated with *Sarcocephaluslatifolius* leaf extract,
17 showed significant decrease ($P < 0.05$) in blood glucose level compared to the untreated rats.
18 Liver biomarkers (ALT, AST and ALP), significantly decreased ($P < 0.05$) in both male and
19 female diabetic rats after treatment with *Sarcocephaluslatifolius* leaf extract compared to the
20 untreated rats. *Sarcocephaluslatifolius* leaf extract has hypolipidemic effect and thus can be
21 used in the management of diabetes.

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

24 **Keywords:***Sarcocephaluslatifolius*,alloxan induced diabetic rats,hypoglycaemia, glucose,
25 lipid profiles, enzyme activity.

26 1. INTRODUCTION

27 Diabetes mellitus is a metabolic disorder that precipitates disturbances in glucose, lipid and
28 protein homeostasis (Van den Bergheet *al.*, 2006). Currently, there is a renewed and growing
29 interest in the use of plant - based products as drugs or as 'leads' in the manufacturing of
30 more potent drugs (Ogbonniaet *al.*, 2008). Several secondary plant metabolites have been
31 shown to modify biological processes, which may reduce the risk of chronic diseases in
32 humans (Ugochukwuet *al.*, 2003) a decoctions of ubulu-inu, Tafashiya or Oya leaves
33 (*sarcocephaluslatifolius*) is used in South Eastern Nigeria in the management of diabetes.
34 Scientific studies also indicated that leaf extract of *sarcocephaluslatifolius* possesses
35 hypoglycaemic effect (Gidadoet *al.*, 2005 and 2008). Diabetes mellitus is the commonest
36 non-communicable endocrine disease and is considered one of the leading causes of death all
37 over the world.

38 The plant African peach (*Sarcocephaluslatifolius*) is of the family Rubiaceae. It is a multi-
39 stemmed tree or shrubup to 12m (Dibuaet *al.* 2013) and the leaves are usually green in
40 colour. It occurs widely throughout Africa. Its generic name is derived from the Greek word
41 sarco (fleshy) and cephalus (headed) in reference to the flowers. The specific epithet is
42 derived from the latin word lati (broad) and folius (leaved). A haemaphrodite tree flowering
43 from April-June and fruits ripen from July-September. Earlier reports on the various
44 medicinal uses of this plant have been reported by many traditional medicine practitioners to
45 be effective in the treatment and management of many ailments such as febrile illness,
46 stomach disorder, cough, malaria fever and jaundices. Others include constipation,
47 dysmenorrhoea abscesses, vomiting and threatened abortions. Clinically *S.latifolius* has been

48 shown to paralyse *Trichostongyluscolumbriformis* larvae in a concentration dependent
49 manner. Distance to health centres, low availability and high costs of modern medicines
50 enhance its patronage.

51 **2. MATERIALS AND METHODS**

52 **2.1. Collection and Identification of Plant Material**

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

57 **2.2. Preparation of Ethanolic Leaf Extract of *Sarcocephalus latifolius***

58 Fresh leaves of *Sarcocephalus latifolius* were dried for three weeks to a constant weight at
59 room temperature under continuous ventilation and reduced to coarse powder with the aid of
60 a mortar and pestle. 60g of the plant's powder was weighed and transferred into 250ml
61 conical flask. This was soaked in absolute ethanol and allowed to stand for 3 days. The
62 content was then shaken on a mechanical shaker for 3 hours and filtered while cold. The
63 filtrate obtained was evaporated to dryness on a water bath. The dry extract was weighed and
64 preserved in a dessicator until when needed. The dry extract was dissolved in distilled water at
65 appropriate concentrations for the various experimental doses using the equation of Tedonget
66 *al.*, 2007.

$$67 \quad V \text{ (ml)} = D \times P/C$$

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

70 **2.3. Phytochemical Screening of the Extract**

71 The ethanolic extract was screened for its phytochemical constituents using standard
72 qualitative procedures (Trease and Evans, 1984; Sofowora, 1986).

73 **2.4. Animals and Biochemical Assays**

74 **2.4.1. Sources of Animals**

75 The study was conducted using twenty four albino wistar rats of mixed sexes (12 males and
76 12 females). They were obtained from the animal house unit of pharmacology Department
77 University of Jos. Stainless steel cages with wire mesh floor were used for housing the
78 animals, free access to water was also allowed alongside grower mesh (locally prepared by
79 the animal farm department of pharmacology university of Jos). They were fed for 2 months
80 to attain a required weight between 150 – 170g.

81 **2.4.2. Administration of Alloxan monohydrate**

82 After 2 months, the rats were divided into six groups of four animals each (3 male groups and
83 3 female groups).
84 Diabetes was induced into the rats in group 1 and 2 for both males and females (as diabetic
85 controls and diabetic treated animals respectively) by injecting them with alloxan
86 monohydrate intraperitoneally with dosage of 150mg/kg body weight while the third groups
87 were used as normal controls. Diabetes was confirmed from the fasting blood glucose after
88 48 hours of induction using on-call plus test strips.

89 **2.4.3. Collection of Blood Samples**

90 After 21 days of treatment with 400mg/kg (body weight) leaf extract, the rats were starved for
91 a night before they were sacrificed by cervical dislocation. The blood samples were collected

92 in clean dry centrifuge tubes and allowed to clot for about one hour and spinned at 300rpm
93 for 10mins. The serum samples were collected and transferred to small clean bottles. The
94 clear supernatant was used for the estimation of Glucose, lipid profile, Alanine transaminase
95 (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP).

96 **2.4.4. Determination of Glucose Level**

97 The glucose concentration was determined using enzymatic indicator test based on the trinder
98 reaction which is quantified by the formation of a pink quinonimine dye.(Trinder, 1969;
99 Teuscher&Richterich, 1971).

100 **2.4.5. Determination of Plasma Lipid Profile**

101 Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoproteins (HDL) were
102 determined using Fortress Diagnostic kits while Low Density Lipoprotein (LDL) was
103 calculated using formula from Friedwald et al.

104 **2.4.6. Determination of Liver Function Test (LFT)**

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

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

112 **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 3.1 below.

Table 3.1

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 3.2a

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

122 **Table 3.2b**

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

123 Values are mean ±S.D

124 3.3 Blood serum Glucose Level

125 The negativecontrol rats showed significant difference (P<0.05) in the blood serum glucose
 126 (mmol/L) when compared with the group of positivecontrol 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 positive control rats as shown in
 129 Table 3.3 below.

130 **Table 3.3**

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

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

137 3.4 Enzyme Activity

138 Table 3.4a and 3.4b shows respectively the female and male aspartate amino transaminase
139 activity (AST), alanine amino transferase activity (ALT), alkaline phosphatase activity (ALP)
140 in the blood serum of the positive and negative control groups.

141 **Table 3.4a**

Female Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
1.Negative 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.Positive control	102.50 $\pm$ 6.87 ^a	73.00 $\pm$ 7.30 ^a	118.50 $\pm$ 1.29 ^b

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

144 **Table 3.4b**

Male Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Negative 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
Positive control	15.00 $\pm$ 2.12 ^a	17.00 $\pm$ 1.47 ^b	33.00 $\pm$ 1.29 ^b

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

147 **3.5 Lipid Profile**

148 The extract reduces the serum levels of cholesterol, triglycerides, Low density lipoproteins
149 (LDL) - cholesterol and High density lipoproteins (HDL) – cholesterol in the treated animals
150 as compared to the untreated ones as shown in Tables 3.5a and 3.5b.

151

152 **Table 3.5a**

Female Group	Cholesterol (mmol/)	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				
+	3.00 ± 0.48 ^a	1.50 ± 0.11 ^a	0.55 ± 0.04 ^b	1.70 ± 0.20 ^b
Leaf extract				
Positive 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 positive control group and
154 treated group with negative control group, b= indicates no significant difference (P>0.05)

155 **Table 3.5b**

Male Group	Cholesterol (mmol/)	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				
+	2.20 ± 0.18 ^b	1.40 ± 0.02 ^b	0.20 ± 0.04 ^a	1.30 ± 0.03 ^a
Leaf extract				
Positive 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 positive control group and
157 treated group with negative 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 *Sarcocephalus latifolius* 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 *Sarcocephalus latifolius*. 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 which
171 shows significant increase in blood sugar level after periods of 21 days of treatment. The
172 result shows that the different concentration of *Sarcocephalus latifolius* extract exhibited a
173 profound reduction ($P < 0.005$) in blood sugar level of the diabetic albino rats. This finding is
174 in line with the results of Rajasekaran *et al.*, 2006 who using both leaf and bark extracts
175 demonstrated the hypoglycaemic effects of the plant. A number of other plants have been
176 reported to have anti-hyperglycemic and insulin stimulator effects.

177 The extract of *Sarcocephalus latifolius* caused significant decrease ($P < 0.05$) in the level of
178 plasma AST, ALT and ALP values in both the female and male rats as shown in Table 3.4a
179 and 3.4b respectively. These indicate that the extract has hepato-protective potentials. This
180 observation is consistent with earlier report on hepatoprotective potentials of the leaf extracts
181 of *V. amygdalin* in mice. The levels of AST, ALT and ALP have been reported to be
182 increased in alloxan-induced diabetic rats. The increase in the activities of AST, ALT and
183 ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into
184 the blood stream, which gives an indication of the hepatotoxic effect of alloxan. The increase
185 in ALT and AST activity in diabetes is always due to hepatocellular damage which may
186 result to leakages into the bloodstream (Sekaret *et al.*, 1990). The reversal of transaminase
187 activity in *Sarcocephalus latifolius* treated diabetic rats towards near normalcy is evidence of

188 prevention cellular and tissue damage under diabetic condition (Rajasekaran, 2006). Also the
189 increase in ALP activity in diabetes is normally due to an injury on the liver.

190 Diabetes induce hyperlipidemia due to excess mobilization of fats from adipose tissue to the
191 under-utilization of glucose. Table 3.5a and 3.5b above, showed that the ethanolic extract of
192 *Sarcocephaluslatifolius* significantly increased the serum HDL – Cholesterol levels while
193 total cholesterol, triglyceride and LDL-Cholesterol decreased significantly ($P<0.05$) in both
194 the female and male diabetic treated groups compared to the untreated groups. The result
195 clearly indicates that the administration of ethanolic leaf extract of
196 *Sarcocephaluslatifolius* produced hypolipidemic effect. This shows that there are many
197 bioactive constituents present in the extract and hence at the present, it is not certain which of
198 them are responsible for the observed effects. Studies have shown that the presence of tannins
199 and flavonoids play an important role in hypolipidemic effect. High levels of triglycerides,
200 LDL-Chol and VLDL-Chol have been associated with heart disease, insulin resistance and
201 diabetic mellitus. Studies have shown that hyperlipidemia is a recognized consequence of
202 diabetes mellitus. In alloxan induced diabetes, the increase in blood glucose level is normally
203 accompanied by an increase in serum Cholesterol, Triglycerides, Low density lipoprotein
204 (LDL) and decrease in high density lipoprotein (HDL) (Mitra *et al.*, 1995) thus excess fatty
205 acid in plasma produce by diabetes promotes the conversion of excess fatty acid into
206 phospholipids and cholesterol in the liver. These two substances along with excess
207 triglycerides formed in the liver may be discharged into blood in the form of lipoproteins
208 (Bopanna *et al.*, 1997).

209 **5. CONCLUSION**

210 The results of this study show that the ethanolic leaf extract of *Sarcocephaluslatifolius* have
211 hepatoprotective actions and suggest that flavonoids present in *Sarcocephaluslatifolius* leaf
212 extract may have a major role in this action. Also other phenolic compounds are known to

213 reduce hyperlipidemia in diabetes. Hence, *Sarcocephaluslatifolius* might be beneficial in the
214 management of diabetes mellitus since it possess hypoglycemic, hypocholesterolemic and
215 hypolipidaemic properties.

216 **CONSENT**

217 Not applicable

218 **ETHICAL APPROVAL**

219 All authors hereby declare that principles of laboratory animal care were followed, as well as
220 specific national laws were applicable. All experiments have been examined and approved by
221 the Departmental Animal Science Ethical committee on the Use and Care of Experimental
222 Animals.

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224 **REFERENCES**

- 225 1. Ugochukwu, N.H., M.K. Cobourne. Modification of Renal Oxidative Stress and Lipid
226 Peroxidation in Streptozotocin-induced Diabetic Rats Treated with Extracts from
227 *Gongronemalatifolium*Leaves. Clin. Chim. Acta. 2003; 336: 73 –81
- 228 2. Trease, G. E. and Evans, W. C.: Textbook of Pharmacognosy. 14th edition. W.
229 Saunders, London. 1989; Pp 46 – 47, 832.
- 230 3. Ugochukwu, N.H., N.E. Babady, M.K. Cobourne and S.R. Gasset. The Effect
231 of *Gongronemalatifolium*Extracts on Serum Lipid Profile and Oxidative Stress
232 inHepatocytes of Diabetic Rats. J. Biosci. 2003; 28(1): 1-5.
- 233 4. Van den Berghe, G., A. Wilmer G. Hermans, W. Meersseman, P.J. Wouters, I.
234 Milants, Van E.Wijngaerden, H. Bobbaers, R. Bouillon. Intensive insulin therapy in
235 the medical ICU. N. Engl. J. Med. 2006; 354(5): 449-461.
- 236 5. Gidado, A., Ameh, D.A., and Atawodi, S.E. Hypoglycemic activity of
237 *Nauclealatifolia* in lowering glucose level in rats.Afri J Biotech. 2005; 4:91- 93.

- 238 6. Dibua, U. M., Kalu, A., Attama, A. A., Esimone, C. O. and Eyo, J. E. In vivo and
 239 invitro evaluation of the inhibitory effect of some medicinal plant extracts on
 240 haemozoin concentration. 2013
- 241 7. Trinder P. Determination of blood glucose using 4-Aminophenazone. J. Clin. Path.
 242 1969; 22: 246
- 243 8. Teuscher A. and Richterich P. Schweiz. Med. Wochenschr. 1971; 101: 342-390.
- 244 9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of
 245 lowdensity lipoprotein cholesterol in plasma, without use of the preparative
 246 ultracentrifuge. Clin; 1972.
- 247
- 248 10. Tedong, L., Dimo, T., Dzeufiet, P.D.D. and Asongalem, A.E., Sokeng D.S., Cal-lard
 249 P., Flejou, J. and Kamtchouing, P. Antihyperglycemic and renal protective activities
 250 of Anacardiumoccidentale (Anacardiaceae) leaves in streptozotocin induced
 251 diabetic rats. African Journal of Traditional, Complementary and Alternative
 252 Medicines. 2007;3(1):23-35
- 253 11. Rajasekaran, S., Ravi, K., Sivagnanam, K., Subramanian, S. Beneficial effects of aloe
 254 vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin.
 255 Exp. Pharmacol. Physiol. 2006; 33, 232–237.
- 256 12. Sekar, N., William, S., Balasubramaniam, N., Kamarajan, P., Govindasamy, S.
 257 Optimization of sodium orthovanadate to treat streptozotocin-induced diabetic rats.
 258 Journal of Biosciences. 1990; 15, 67-75.
- 259 13. Mitra, A and Bhattacharya, D.: Effects of overall consumption, dietary
 260 patterns,cooking, on patients suffering from Non insulin dependent diabetes mellitus.
 261 J of Interacad. 2005; 9 (4): 635-642.

262 14. Bopanna K.N., Kannan J., Sushma G., Balaraman R. and Rathod S.P.:
263 Antidiabetic and antihyperlipidemic effects of neem seed kernel powder on alloxan
264 diabetic rabbits. Indian Journal of Pharmacology. 1997; 29: 162–167.