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ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *CALOTROPIS PROCERA* LEAF IN ALLOXAN-INDUCED DIABETIC RATS

[1*]Ajiboso S. O., [2]Yakubu T. M and [3]Oladiji A. T

[1*]Biochemistry Research Laboratory, Science Laboratory Technology Department, The Federal Polytechnic, P. M. B 55 Bida, Niger State, Nigeria.
[2,3]Biochemistry Department, University of Ilorin, P.M.B 1515 Ilorin, Kwara State, Nigeria.

***Author for Correspondence: Ajiboso S. O.**
Biochemistry Research Laboratory, Science Laboratory Technology Department, The Federal Polytechnic, P. M. B 55 Bida, Niger State, Nigeria.,

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ABSTRACT

Aqueous extract of *Calotropis procera* leaf was investigated for antidiabetic activity in alloxan-induced diabetic rats. Adult Wistar rats of mean weight 110.0±1.4 g were randomised into six groups (A-F) such that group A (non-diabetic) received orally 0.5ml of distlled water once daily for 10 days. Animals in group B, C, D, E and F which were made diabetic with alloxan (150 mg/kg body weight i.p) also received once daily 0.5ml of metformin (2.5 mg/kg b.w p.o), 25, 50 and 100 mg/kg body weight o.p. of the extract respectively. Standard procedures were used to determine blood glucose, body weight, oral glucose tolerance test, weight of pancreas, serum insulin, total protein, urine glucose and ketone. The results of the antidiabetic study revealed that blood glucose of the alloxanised rats within 36 hours were significantly and progressively reduced ($p<0.05$) in the metformin - and extract - treated animals. The trace amount of urine ketone and glucose concentrations in the distilled water - treated diabetic rats were not observed in the other treatment groups except in the 25 mg/kg body weight extract treated group where less than 0.25 % of the urine glucose concentration was observed. The reduction in body weight, weight of pancreas, serum insulin and total protein in the distilled water-treated diabetic rats were reverted back to the range of the non-diabetic controls by the extract and metformin. The significant elevation ($p>0.05$) of oral glucose in distilled water treated diabetic rats was also reverted back to normal. The present study thus provides scientific evidence to support the acclaimed use of *Calotropis procera* leaf for the treatment of diabetes mellitus in folk medicine in Nigeria.

KEYWORDS: *Calotropis procera*, antidiabetic activity.

INTRODUCTION

Diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (World Health Organization, 2005). In 2000, the incidence of diabetes in Africa was expected to increase from 9.4 million to 14.1 million in the year 2010 (Zimmet *et al.*, 2001). The estimated prevalence of diabetes in Africa is 1 % in rural areas, up to 5 % to 7 % in urban sub-Saharan Africa, and between 8 % and 13 % in more developed countries such as South Africa and in populations of Indian origin (Motala *et al.*, 2003).

Diabetes is becoming the third 'killer' disease of mankind, after cancer and cardiovascular diseases because of its high prevalence, morbidity and mortality (Li *et al.*, 2004). According to the 2004 estimates, the Diabetes Association of Nigeria (DAN) puts the diabetics' population in Nigeria at about 1 million (Ogbera *et al.*, 2005). However an estimate for diabetics' population (age 20-79) in Nigeria for 2010 was put at 2,819,000 and was projected to rise up to 5,316,000 in 2030 while the calculated mean annual increment was put at 125,000 (Okoro and Ogbera, 2013).

Currently, there are several drugs available for treatment of diabetes mellitus but most of them are synthetic drugs and this makes the anti-diabetic treatment costly. In view of reducing the cost of treatment for diabetes mellitus, there is considerable research for herbal production as anti-diabetics and around 12,000 plants have been reported to possess antidiabetic property.

There are claims on the use of *Calotropis procera* leaves by folklore tradition of Nigeria in management of several ailments, including diabetes. Such claim on the use of *Calotropis procera* leaves as antidiabetic agent is yet to be substantiated with scientific experiment. Therefore,

this research work was designed to evaluate the acclaimed use of *Calotropis procera* leaves as antidiabetic agent.

MATERIALS AND METHODS

Experimental animals

Adult Wistar rats (both sex) of mean weight 110.0±1.4 g obtained from the animal house of the Biochemistry Department, University of Ilorin, Ilorin, Kwara state, Nigeria were used for the study. The animals were fed on rat basal diet (Vital GCOML), throughout the period of the experiment.

Collection and authentication of plant sample

Matured fresh leaves of *Calotropis procera* were collected from the botanical garden of the Federal Polytechnic, Bida, Niger State in March, 2012 and were authenticated at the Plant Biology Section, Federal Polytechnic, Bida, Niger State, Nigeria, where a voucher specimen (No. 94067) was deposited at the herbarium Unit.

Glucometer and Assay kit

Bayer Contour^{TM} TS blood glucose kit was a product of Bayer Consumer Care AG, Postfash, Basel, Switzerland while combi-5 kit for urinalysis was a product of Meditex, Regensburg, Germany.

Drug and Chemicals

Alloxan monohydrate was a product of Explicit Chemicals PVT, Ltd., Pune, India. while Metformin was a product of NWP Springville, Illinois, USA. All other chemicals were products of Sigma-Aldrich CHEME Gmbh, Steinhelm Germany. The chemicals were prepared in glass distilled water unless otherwise stated.

METHODS

Preparation of Extract

The method described by Yakubu *et al.,* (2010) was used to prepare the extract.

Induction of diabetes

The method described by Yakubu *et al.,* (2010) was used to induce diabetes. The blood glucose levels of the rats were determined before the administration of alloxan. Only animals with blood glucose level higher than 11.1 mmol/L were used for the study.

Animal grouping and Extract administration

30 rats (5 normal, 25 alloxan induced-diabetic rats) were distributed into six groups (A-F) of five rats each after diabetes had been confirmed.

A= Non-diabetic rats given 0.5 ml of distilled water
B= Diabetic untreated rats administered 0.5 ml of distilled water
C= Diabetic rats administered 0.5 ml of 2.5 mg / kg body weight of Metformin
D= Diabetic rats administered 0.5 ml of 25 mg / kg body weight of extract.
E= Diabetic rats administered 0.5 ml of 50 mg / kg body weight of extract.
F= Diabetic rats administered 0.5 ml of 100 mg/ kg body weight of extract.

Calculated amount of the residue was weighed and constituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight. The doses used in this study were as obtained from the ethno-botanical survey carried out on the plant within our locality. Treatment was administered orally with feeding bottle to respective groups. Preliminary studies conducted by Yakubu *et al.* (2010) revealed that the diabetic untreated rats could survive up till the 12th day; therefore this experiment was terminated on the 10th day. The rats were handled in accordance with the guidelines of European Convention for the protection of vertebrate animals and other scientific purposes -ETS-123 (ETS, 2005).

Determination of blood glucose and body weight

Blood glucose levels and body weights of each rat were determined on days 0, 5 and 10 using glucometer (Bayer Contour^{TM} AG, Postfash, Basel, Switzerland) and digital electronic weighing balance (Gilbertini, Italy) respectively.

Oral Glucose Tolerance Test

The procedure described by Eseyin *et al*., (2010) was used to determine the oral glucose tolerance test.

Determination of Urine glucose and ketone

The method described by Yakubu *et a.*, (2010) was used to determine urine glucose and ketone concentration.

Preparation of serum and tissue homogenate

Determination of weight of Pancreas

The weight of the pancreas was calculated as follows:

$W2 = W1 – W0$

Where:

W2 =Weight of Pancreas
W1 = Weight of Pancreas + Petri dish
W0 = Weight of Petri dish

Determination of some biochemical parameters

Determination of serum insulin concentration

Serum insulin concentration was determined according to the procedure described by Lygren *et al*., (2013).

Determination of Total Protein concentration

Total protein concentration was determined according to the procedure described by Gomall *et al.,* (1949).

RESULTS

Table 1: Effects of administration of aqueous extract of *Calotropis procera* leaf on blood glucose level of diabetic rats Blood glucose (mmol/L)

Group / Day	0	5	10
Non- diabetic+Distilled water	3.30±0.10[a]	3.40±0.00[a] (3.03%)	3.40±0.10[a] (3.03%)
Diabetic rats +Distilled water	22.70±0.30 [c]	27.40±2.00 [b] (-20.71%)	32.70±1.00[b] (-44.05%)
Diabetic rats +Metformin	20.70±3.10[d]	7.80±0.10[e] (62.32%)	3.40±0.00[a] (83.58%)
Diabetic rats + 25mg/kg body weight of the extract	22.90±1.00[c]	21.60±3.00[c] (5.68%)	19.90±1.70 [c] (13.10%)
Diabetic rats + 50mg/kg body weight of the extract	23.20±0.00[c]	16.20±0.10[d] (30.17%)	9.70±0.30[d] (58.19%)
Diabetic rats + 100mg/kg body weight of the extract	25.70±1.00[b]	8.50±0.00 [c] (66.92%)	3.90±0.00[a] (84.83%)

Values are Means + SEM of 5 determinations; Values down each column carrying different superscript are significantly different ($p<0.05$) from non-diabetic control, percentages in parenthesis are levels of reduction and elevation of blood glucose; - = percentage reduction of blood glucose.

Table 2: Effects of administration of aqueous extract of *Calotropis procera* leaf on oral glucose tolerance of diabetic rats.

Group / Time (minutes)	0	15	Blood glucose (mmol/L) 30	45	60
Non- diabetic+Distilled water	4.80±0.00[a]	8.10 ±0.00[a]	7.60 ±0.30[a]	5.00 ±0.10[a]	4.20 ±0.00[a]
Diabetic rats +Distilled water	18.70 ±1.00 [b]	27.20 ±1.00[b]	23.20 ±3.20[b]	22.40 ±2.00[b]	22.00 ±0.00[b]
Diabetic rats +Metformin	17.00 ±0.20[b]	20.10 ±0.10[c]	14.10 ±1.10[d]	12.30 ±0.00[d]	11.70±1.30[e]
Diabetic rats + 25mg/kg body weight of the extract	18.70 ±3.10[b]	22.30 ±1.70[c]	21.50 ±0.00[b]	20.90 ±0.10[b]	17.40 ±1.00[c]
Diabetic rats + 50mg/kg body weight of the extract	18.10 ±2.00[b]	20.40 ±0.10[c]	18.30 ±0.00[c]	16.20 ±0.00[c]	14.10 ±0.10[d]
Diabetic rats + 100mg/kg body weight of the extract	18.00 ±0.70 [b]	22.10 ±1.00[c]	15.70 ±0.10[d]	13.10 ±0.50[d]	11.10 ±1.00[e]

Values are Means + SEM of 5 determinations; Values down the column carrying different superscript are significantly different ($p<0.05$) from non-diabetic control

Table 3: Effects of administration of aqueous extract of *Calotropis procera* leaf on urine glucose content of diabetic rats Day after administration of alloxan.

Group	0	% 5	10
Non- diabetic+Distilled water	ND	ND	ND
Diabetic rats +Distilled water	>2	>2	>2
Diabetic rats +Metformin	>2	0.25	ND
Diabetic rats + 25mg/kg body weight of the extract	>2	>0.25<1	0.25
Diabetic rats + 50mg/kg body weight of the extract	>2	0.25	ND
Diabetic rats + 100mg/kg body weight of the extract	>2	ND	ND

Values are Means + SEM of 5 determinations

ND= Not detected; (+)= 0.25%; (++)= more than 0.25% but not up to 1%; (+++)= more than 2%

Table 4: Effects of administration of aqueous extract of *Calotropis procera* leaf on urine ketone content of diabetic rats.

Day after administration of alloxan.

Group	0	% 5	10
Non- diabetic+Distilled water	ND	ND	ND
Diabetic rats +Distilled water	>0.25<1.00	>0.25<1.00	>0.25<1.00
Diabetic rats +Metformin	0.25	ND	ND
Diabetic rats + 25mg/kg body weight of the extract	0.25	ND	ND
Diabetic rats + 50mg/kg body	0.25	ND	ND

weight of the extract			
Diabetic rats + 100mg/kg body weight of the extract	0.25	ND	ND

Values are Means + SEM of 5 determinations
ND= Not detected; (+)= 0.25%; (++)= more than 0.25% but not up to 1%.

Table 5: Effects of administration of aqueous extract of *Calotropis procera* leaf on body weight of diabetic rats Day after administration of alloxan.

Group	0	(g) 5	10
Non- diabetic+Distilled water	110.30±7.10[a]	115.30±3.50[a] (4.53%)	122.00±2.00[a] (10.61%)
Diabetic rats +Distilled water	110.50±1.00[a]	97.20±1.00[c] (-12.04%)	90.30±0.00[c] (-18.28%)
Diabetic rats +Metformin	110.00±4.20[a]	107.30±2.10[b] (2.46%)	116.50±1.00[b] (5.91%)
Diabetic rats + 25mg/kg body weight of the extract	109.10±3.70[a]	106.50±3.00[b] (2.38%)	117.30±1.00[b] (7.52%)
Diabetic rats + 50mg/kg b Ody weight of the extract	110.00±4.80[a]	107.20±1.00[b] (2.55%)	117.50±3.00[b] (8.64%)
Diabetic rats + 100mg/kg body weight of the extract	110.00±5.00[a]	106.30±0.00[b] (3.36%)	121.20±4.20[a] (10.18%)

Values are Means + SEM of 5 determinations
Values down each column carrying different superscript are significantly different ($p<0.05$) from non-diabetic control percentages in parenthesis are levels of reduction and elevation of blood glucose.

Table 6: Effects of administration of aqueous extract of *Calotropis procera* leaf on some biochemical parameters of diabetic rats.

Group	Weight of Pancreas (g)	Serum insulin (µU/ml)	Serum total protein (mg/dl)
Non- diabetic + Distilled Water	0.80±0.0 0[a]	19.70±1.70[a]	71.40±0.20[a]
Diabetic rats + Distilled Water	0.30±0.00[b]	6.20±1.00[d]	39.10±2.30[d]
Diabetic rats +Metformin	0.80±0.00[a]	19.10±0.10[a]	70.50±4.00[a]
Diabetic rats + 25mg/kg body weight of the extract	0.70±0.00[a]	10.30±1.10[c]	45.80±1.20[c]
Diabetic rats + 50mg/kg body weight of the extract	0.70±0.00[a]	15.90±0.10[b]	61.40±1.00[b]
Diabetic rats + 100mg/kg body weight of the extract	0.80±0.00[a]	19.30±1.30[a]	70.90±2.20[a]

Values are Means + SEM of 5 determinations
Values down each column carrying different superscript are significantly different ($p<0.05$) from non-diabetic control.

Effects of administration of aqueous extract of *Calotropis procera* leaf on blood glucose of diabetic rats

The effects of administration of aqueous extract of *Calotropis procera* leaf on blood glucose level of diabetic rats are shown in Table 1. The result revealed that all the alloxanised diabetic rats showed significant increase ($p<0.05$) in blood glucose after 36 hours with blood glucose levels ranging from 20.70 - 25.70 mmol/L. The blood glucose levels were however reduced significantly ($p<0.05$) and progressively in the extract - and metformin - treated rats. By the end of the treatment, the extract at the doses of 25, 50 and 100 mg/kg body weight had reduced the blood glucose levels of the rats by 13.10 %, 59.19 % and 84.83 % respectively. The percentage reduction in the blood glucose level caused by 100 mg/kg body weight of the extract in treated rats was similar to the 83.58 % reduction obtained for the metformin - treated rats and showed no significant difference ($p > 0.05$) compared to non-diabetic control.

Effects of administration of aqueous extract of *Calotropis procera* leaf on oral glucose tolerance of diabetic rats

The effects of administration of aqueous extract of *Calotropis procera* leaf on oral glucose tolerance of diabetic rats are presented in Table 2. The result revealed a significant increase ($p<0.05$) in blood glucose levels of the alloxanised diabetic rats 15 minutes after oral glucose administration. A significant reduction ($p<0.05$) in blood glucose levels was recorded in the extract - treated rats 30 minutes after oral glucose administration, the

reduction in blood glucose was progressive till 60 minutes post glucose load. The mean blood glucose value of 11.10 mmol / L obtained in rats treated with 100 mg/kg body weight was similar to the mean blood glucose value of 11.70 mmol / L obtained in the metformin - treated rats at 60 minutes post glucose load. There was no significant difference ($p>0.05$) in the oral glucose tolerance of of rats treated with 100 mg/kg body weight of the extract and metformin.

Effects of administration of aqueous extract of *Calotropis procera* leaf on urine glucose concentration of diabetic rats

The effects of administration of aqueous extract of *Calotropis procera* leaf on urine glucose concentration of diabetic rats are shown in Table 3. The result revealed that high glucose concentration (above 2 %) was detected in the urine of distilled water - treated diabetic rats and sustained throughout the treatment period, whereas similar urine glucose concentration (above 2 %) obtained in the other treatment groups on day 0 disappeared while the study lasted, except in rats treated with 25 mg/kg body weight of the extract where trace amount of 0.25 % urine glucose was detected.

Effects of administration of aqueous extract of *Calotropis procera* leaf on urine ketone content of diabetic rats

The effects of administration of aqueous extract of *Calotropis procera* leaf on urine ketone content of diabetic rats are presented in Table 4. The result revealed that high amount of ketone (more than 0.25 % but less than 1 %) was detected in the distilled water - treated diabetic rats and sustained throughout the experimental period. However, the trace amount of 0.25 % of ketone in the other treatment groups observed on day 0, was not detected in the urine of extract - and metformin - treated rats from day 5 till day 10.

Effects of administration of aqueous extract of *Calotropis procera* leaf on body weight of diabetic rats

The effects of administration of aqueous extract of *Calotropis procera* leaf on body weight of diabetic rats is presented in Table 5. The result revealed a significant reduction ($p<0.05$) in body weight of distilled water - treated diabetic rats when compared to non-diabetic rats; which showed significant increase ($p<0.05$) in body weight throughout the treatment period. By the end of the 10-day treatment, the extract at the doses of 25, 50 and 100 mg/kg body weight had increased the body weight of the treated rats by 7.52 %, 8.64 % and 10.18 % respectively. There was no significant difference ($p>0.05$) in percentage increase in (10.18 %) in body weight of rats treated with 100 mg/kg body weight of the extract and that of the non-diabetic rats (10.91 %).

Effects of administration of aqueous extract of *Calotropis procera* leaf on some biochemical parameters of diabetic rats

The effects of administration of aqueous extract of *Calotropis procera* leaf on some biochemical parameters in diabetic rats are presented in Table 6. The result showed significant decrease ($p<0.05$) in weight of pancreas, serum insulin and total protein in distilled water - treated diabetic rats when compared to non-diabetic rats. Treatment with the extract normalised the altered parameters. There was no significant difference ($p > 0.05$) in values obtained for all the parameters in groups treated with 100 mg/kg body weight of the extract and metformin treated rats.

DISCUSSION

Effects of administration of aqueous extract of *Calotropis procera* leaf on blood glucose of diabetic rats

Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The use of a low dose of alloxan monohydrate (150 mg/kg body weight) produced an incomplete destruction of pancreatic β-cells, leading to poor production of insulin for glucose uptake by tissues even though the rats became permanently diabetic (Aybar *et al.*, 2002). Alloxan-induced diabetes has been described as a useful experimental model to study the anti-diabetic activity of several agents (Papaccio *et al.*, 2000). Alloxan is well known for its selective pancreatic Islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Sezik *et al.*, 2005). In the present study, the alloxan dose (150 mg/kg body weight) was selected in order to partially destroy the pancreatic β-cells. Under this condition, insulin was secreted, but not in sufficient amount to regulate blood glucose levels, consequently the rats became permanently diabetic. Intraperitoneal administration of alloxan (150 mg/kg body weight) effectively induced diabetes in normal rats, as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and bodyweight loss compared with normal rats in this present study (Papaccio *et al.*, 2000). The increased levels of blood glucose in alloxan-induced diabetic rats were lowered by the administration of the extract suggesting that the extract might be exerting insulin-like effect on peripheral tissues by promoting glucose uptake, stimulation of a regeneration process (Bolkent *et al.*, 2000) and revitalisation of the remaining β-cells (Rokeya *et al.*, 2005) or inhibiting hepatic gluconeogenesis and by absorption of glucose into the muscle and adipose tissues (Gray *et al.*, 2000).

Effects of administration of aqueous extract of *Calotropis procera* leaf on oral glucose tolerance of diabetic rats

The oral glucose tolerance test (OGTT) is a widely used procedure in the diagnosis of diabetes and intermediate stages of hyperglycemia (Anita *et al.*, 2012). It measures the body's ability to use glucose which is the body's main

source of energy (Eseyin *et al.,* 2010). OGTT, is also a test of immense value, which is seen as a practical attempt to simplify and facilitate the diagnosis of diabetes (Eseyin *et al.,* 2010). The present study showed an appreciable improvement in glucose tolerance which could be attributed to the insulinotropic activity of the extract by restoring the insulin response. Hence, it was confirmed that 100 mg/kg body weight of the extract showed the highest percentage of glycemic index in the treated rats and higher glycemic control than other doses of the extract. The observation on glycemic control by the extract made in the present study confirms the effect on glucose tolerance test (GTT) and metabolic profile in early and late stages in diabetic rats.

Effects of administration of aqueous extract of *Calotropis procera* leaf on urine glucose concentration of diabetic rats

Presence of glucose in the urine suggests excretion of blood glucose that is above renal threshold into the urine, a condition known as glycosuria (Komoroski *et al.,* 2008). Glycosuria is most commonly due to untreated diabetes mellitus and leads to excessive water loss into the urine with resultant dehydration, a process called osmotic diuresis (Onyemelukwe and Bakari, 2002). The presence of excess glucose in the urine of diabetic control rats confirms a problem with glucose reabsorption within the kidneys. Dehydration is also evident in the distilled water - treated diabetic rats. However, the clearance of the glucose in the urine of the treated groups offered by the extract agrees with the report of Yakubu *et al.,* (2010) on anti-diabetic activity of aqueous extract of *Cochlospermum planchonii* root in alloxan-induced diabetic rats.

Effects of administration of aqueous extract of *Calotropis procera* leaf on urine ketone concentration of diabetic rats

Elevated blood ketone level has been reported to be a common feature of diabetes mellitus (Noor *et al.,* 2008) and this has been further corroborated in the present study by the presence of significant amount of urine ketone in the alloxanised diabetic rats. Although, the presence of ketone may be attributed to enhanced production of ketone bodies by the liver and incomplete utilisation by the tissue leading to accumulation in the blood and subsequent elimination in the urine (Wadkar *et al.,* 2008). The ability of the aqueous extract of *Calotropis procera* leaf to ameliorate the elevated urine ketone level in the treated diabetic rats, in a manner similar to metformin, did not only buttress the anti-diabetic potential of the extract but also its effectiveness against this metabolic disorder that is characteristic of diabetes mellitus. The detection of urine ketone in the urine of diabetic rats in the present study is in accordance with the findings of Yakubu *et al.,* (2010) on anti-diabetic activity of aqueous extract of *Cochlospermum planchonii* root in alloxan-induced diabetic rats.

Effects of administration of aqueous extract of *Calotropis procera* leaf on body weight of diabetic rats

In distilled water - treated diabetic rats, the characteristic loss in body weight could be due to poor glycemic control leading to an increase in muscle wasting (Vats *et al.,* 2004). The excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency results in muscle wasting and weight loss in distilled water - treated diabetic rats (Vats *et al.,* 2004). The loss in body weight in distilled water treated - diabetic rats could be attributed to defect in glucose uptake and metabolism (Sezik *et al.,* 2005). Improvement in body weight in extract - treated rats may be attributed to the crude protein and fat contents of the extract. The crude protein content of the extract may play a compensatory role by replacing lost protein resulting from proteolysis and muscle wasting syndrome (Vats *et al.,* 2004). The fat content of the extract may also increase the appetite of the treated rats and thus attenuate anorexia due to the ability of fat to absorb and retain flavours (Akubugwo, 2007).

Effects of administration of aqueous extract of *Calotropis procera* leaf on some biochemical parameters in diabetic rats

The reductions in the weight of pancreas of distilled water - treated diabetic rats in the present study may be attributed to decrease in number of secretory granules of the β-cells of the pancreas. Kumar *et al.,* (2006) reported a decrease in the number of secretory granules of the β-cells of the pancreas in diabetic rats. It is possible that the normal architecture of this secretory granules were restored following the administration of aqueous extract of *Calotropis procera* leaves and metformin since the weight of the pancreas in these groups compared favourably with non-diabetic group. According to Campbell-Thompson (2012), the weight of pancreas in individuals with serum markers that potentially precede the clinical manifestations of type 1 diabetes, was less than in controls. This suggests that early atrophy of the organ may be an important sub-clinical feature of type 1 diabetes pathogenesis (Yakubu *et al.* 2010). Furthermore, the reductions in weight of pancreas of the diabetic rats in this study confirm the earlier reports of Campbell-Thompson (2012) and Yakubu *et al.,* (2010). Insulin is a hormone secreted in times of ample glucose supply and promotes glucose utilization. Its major metabolic effects include stimulation of glycolysis, glycogen synthesis and triacylglycerol synthesis and inhibition of gluconeogenesis, glycogenolysis and triacylglycerol breakdown (Davis and Granner, 2001). Insulin also controls the uptake of glucose into the cells of many tissues such as the muscles and adipose tissues. The effects of alloxan on glucose and insulin homeostasis reflect the toxin-induced abnormalities in β-cell function (Strandell *et al.,* 1988). Metformin stimulates insulin secretion from pancreatic β-cells (Davis and Granner, 2001). Based on these results, it can be hypothesized that aqueous extract of *Calotropis procera* leaf may probably act by releasing insulin from pancreatic β-cells through

insulin synthesis and secretion (Venkateswaran and Pari, 2003). The reduced protein content in the sera of diabetic rats was reported by Changrani *et al.*, (2006). The reduced protein content of distilled water - treated diabetic rats agree with the reports of Changrani *et al.*, (2006).

CONCLUSION

Antidiabetic activity of aqueous extract of *Calotropis procera* leaf in alloxan-induced diabetic rats was carried out in this study. This study revealed that daily oral administration of the aqueous extract of *Calotropis procera* leaf particularly at the dose of 100 mg/kg body weight to diabetic rats produced anti-hyperglycemic effect that was comparable to that of metformin (2.5 mg/kg body weight) within 10 days of administration. Aqueous extract of *Calotropis procera* leaf also produced a marked reduction in the levels of urinary ketone and glucose. In conclusion, the present study thus provides scientific evidence to support the acclaimed use of Aqueou**s** extract of *Calotropis procera* leaf and justify the use of 100 mg/kg body weight dose of the extract for the treatment of diabetes mellitus in the folk medicine of Nigeria.

RECOMMENDATIONS

Since there is a growing search for natural products that could be used in the treatment of diabetes, this study provides information on the possible usage of aqueous extract of *Calotropis procera* leaf as an anti-diabetic agent. Further study should be conducted to isolate and purify the bioactive compounds in the aqueous extract of *Calotropis procera* leaf that exert the anti-diabetic properties offered by the plant.

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