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EVALUATION OF HYPOGLYCAEMIC AND ANTIOXIDANT ACTIVITIES OF *CALOTROPIS PROCERA* LEAF IN ALLOXAN-INDUCED DIABETIC RATS

***[1]Ajiboso S. O. and [2]Tarfa F. D.**

[1]Biochemistry Research Laboratory, Department of Chemical Sciences, Federal Polytechnic, PMB 55 Bida, Niger State, Nigeria.

[2]National Institute for Pharmaceutical Research and Development, Idu PMB 21 Garki Abuja Nigeria.

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***Corresponding Author**
Ajiboso S. O.
Biochemistry Research Laboratory, Department of Chemical Sciences, Federal Polytechnic, PMB 55 Bida, Niger State, Nigeria.

ABSTRACT

The hypoglycemic and antioxidant activities of aqueous extract of *Calotropis procera* leaf in alloxan-induced diabetic rats were evaluated in this study. Adult Wistar rats of mean weight 110.0±1.4g were randomised into six groups (A-F), group A (non-diabetic) received orally 0.5ml of distilled water once daily for 10 days, groups B, C, D, E and F were made diabetic with alloxan (150 mg/kg body weight i.p). Groups C, D, E and F 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, hepatic ascorbic acid, reduced glutathione and activities of catalase, glutathione peroxidase and superoxide dismutase. The results obtained revealed significant elevation ($p<0.05$) of blood glucose, liver MDA and reduction ($p<0.05$) of hepatic ascorbic acid content, reduced glutathione level, body weight and activities of catalase, glutathione peroxidase and superoxide dismutase in distilled water-treated diabetic rats. The increased and reduced parameters were reverted back to the range of the non-diabetic control animals in the groups treated with metformin and extract particularly 100mg/kg b.w. dosage.

INTRODUCTION

The global number of individuals with diabetes was expected to project from 171 million (2.8 % of the world's population) in year 2000 to 366 million (6.5%) in 2030; with 298 million

living in developing countries (Wild *et al.*, 2004). International Diabetes Federation (2009) also estimated that another 314 million people have impaired glucose tolerance and that the number will increase to 472 million by 2030. The countries with the largest number of diabetes include India, China and the United States (International Diabetes Federation, 2009).

The search for diabetes in an individual is often driven by the presence of characteristic symptoms such as thirst, polyuria, weight loss, recurrent infections and, in more severe cases, coma. In such individuals, a single elevated casual plasma glucose value is sufficient to confirm diagnosis. Blood glucose levels are normally maintained within a range of 70 to 120 mg/dL (World Health Organisation, 2002).

Oxidative stress results from increased reactive oxygen species (ROS) and reactive nitrogen species (RNS), examples of ROS include charged species such as superoxide and hydroxyl radical, and uncharged species such as hydrogen peroxide and singlet oxygen. The possible sources of oxidative stress in diabetes might include auto-oxidation of glucose, shifts in redox balances, decreased tissue concentrations of low molecular weight antioxidants, such as reduced glutathione (GSH) and vitamin E, and impaired activities of antioxidant defence enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Haskins *et al.*, 2003). ROS generated by high glucose is causally linked to elevated glucose and other metabolic abnormalities to the development of diabetic complications. However, the exact mechanism by which oxidative stress may contribute to the development of diabetic complications is undetermined (Kowluru and Chan, 2007).

Calotropis procera (Sodom apple- English; Bomubomu- Yoruba; Tumfafiya- Hausa; Epuko- Nupe) (Ajagbonna *et al.*, 1999), is a wild growing plant of *Asclepiadaceae* family. In India, the plant is known as Madar in Hindi, Orka in Oriya, and Alarka in Sanskrit (Meena *et al.*, 2011). In the traditional Indian medicinal system, it has been used for the treatment of leprosy, ulcers, tumors, piles, diseases of spleen, liver and abdomen (Larhsini *et al.*, 1997). The aqueous extract of the flower has been shown to possess analgesic, antipyretic and anti-inflammatory activities (Dewan *et al.*, 2000).

In Nigeria, the plant is well known for its medicinal properties, different parts of this plant have been reported to exhibit anti-inflammatory and analgesic properties (Ajagbonna *et al.*, 1999).

The objective of this study was to evaluate the hypoglycemic and antioxidant activities of aqueous extract of *Calotropis procera* leaf in alloxan-induced diabetic rats through the determination of its effects on body weight and some biochemical markers such as blood glucose level, liver MDA, non-enzymatic antioxidants (reduced glutathione, ascorbic acid) and activities of enzymatic antioxidants (catalase, superoxide dismutase, glutathione peroxidase).

MATERIALS

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.

Glucometer and Assay kit

Bayer Contour^{TM} TS blood glucose kit was a product of Bayer Consumer Care AG, Postfash, Basel, Switzerland. Assay kits for total cholesterol, triacylglycerol and HDL-cholesterol were products of Randox Laboratories, Co-Antrim, UK.

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. Fresh leaves of *Calotropis procera* were cut into very thin slices, air dried at room temperature for 72

hours to a constant weight. The dried materials were pulverized using an electric blender (Phillips Comfort, model HR 1727, Holland). A known weight of the powder (280 g) was extracted in 500 ml of distilled water for 12 hours with intermittent shaking. The extract was filtered with Whatman No. 1 filter paper and thereafter evaporated on a lyophilizer at 55^{o}C for 30 minutes to give a yield of 7.98 g.

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.

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. 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 to groups D, E and F respectively while groups A, B and C were non-diabetic, distilled water treated diabetic and metformin treated diabetic rats respectively. 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 (European Treaty Series, 2005).

Determination of blood glucose and body weight

Blood glucose level of each rat was determined on days 0, 5 and 10 with the aid of glucometer (Bayer Contour^{TM} AG, Postfash, Basel, Switzerland).

Preparation of tissue homogenate

The procedure described by Yakubu *et al*., (2010) was used to prepare tissue homogenate.

At the end of 10th day, under anaesthesia (diethyl ether, 50 mg / ml), the rats were quickly dissected and the livers were removed. The liver was cut into tiny pieces and homogenised 0.25M sucrose solution (1: 5 w/v) using hand-held homogenizer (model D1000 Asteria Inc.

New Jersey, USA). The homogenates were immediately transferred into specimen bottles and kept frozen for 24 hours before analysis.

Determination of Malondialdehyde concentration and Antioxidant status

The procedure described by Varshney and Kale (1990) was used to determine malondialdehyde concentration in liver homogenates of the experimental rats. The catalase (CAT) activity was determined using the procedure described by Abei (1988). The Superoxide Dismutase (SOD) activity was determined according to the procedure described by Marklund and Marklund (1974). Glutathione peroxidase activity was determined according to the procedure described by Paglia and Valentine (1967). Reduced glutathione (GSH) content was determined according to the procedure described by Griffith (1980). Ascorbic acid content was determined according to the procedures described by Yijing *et al.*, (1999) and Okamura (1980).

RESULTS

Table 1: Effect 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	1.31 ± 0.10^{a}	1.42 ± 0.10^{a}	2.40 ± 0.10^{a}
Diabetic rats +Distilled water	12.30 ± 0.30^{c}	17.41 ± 1.00^{d}	22.70 ± 1.00^{c}
Diabetic rats +Metformin	10.20 ± 3.10^{b}	6.24 ± 0.00^{b}	2.40 ± 0.00^{a}
Diabetic rats + 25mg/kg body weight of the extract	12.00 ± 1.00^{c}	11.61 ± 0.00^{c}	9.10 ± 1.70^{b}
Diabetic rats + 50mg/kg body weight of the extract	13.30 ± 0.00^{d}	10.90 ± 0.20^{c}	8.70 ± 0.30^{b}
Diabetic rats + 100mg/kg body weight of the extract	14.20 ± 1.00^{e}	6.80 ± 0.30^{b}	2.60 ± 0.00^{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

Effect of administration of aqueous extract of *Calotropis procera* leaf on blood glucose level of diabetic rats was determined in this study. The results were presented in Table 1, blood glucose of the experimental rats was determined before induction of diabetes (day 0) and after induction (days 5 and 10).

The untreated diabetic rats administered distilled water only throughout the period of experiment showed significant increase ($p<0.05$) in blood glucose levels from 12.30mmol/L on day 0 to 22.70mmol/L on day 10.

The positive control group (non-diabetic rats administered distilled water) showed blood glucose range of 1.31mmol/L to 2.40mmol/L from days 0 to 10 respectively. Diabetic rats treated with metformin and extracts showed significant decrease ($p<0.05$) in blood glucose levels.

The hypoglycaemic performance of the extracts was dose-dependent, 25mg/kg b.w. extract showed the least performance while 100mg/kg b.w. extract showed the highest performance and this compared favourably with the performance of the standard hypoglycaemic drug – metformin.

Table 2: Effect of administration of aqueous extract of *Calotropis procera* leaf on body weight of diabetic rats.

	Day after administration of alloxan		
Group/ body weight (g)	**0**	**5**	**10**
Non- diabetic+Distilled water	110.10±7.10^{a}	117.30±3.50^{a}	120.00±2.00^{a}
Diabetic rats +Distilled water	110.30±1.00^{a}	95.20±1.00^{c}	87.30±0.00^{d}
Diabetic rats +Metformin	110.20±4.20^{a}	106.30±2.10^{b}	119.50±1.00^{c}
Diabetic rats + 25mg/kg body weight of the extract	110.00±3.70^{a}	105.50±3.00^{b}	115.30±1.00^{c}
Diabetic rats + 50mg/kg body weight of the extract	110.30±4.80^{a}	106.20±1.00^{b}	118.50±3.00^{b}
Diabetic rats + 100mg/kg body weight of the extract	110.20±5.00^{a}	105.30±0.00^{b}	120.20±4.20^{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.

Effect of administration of aqueous extract of *Calotropis procera* leaf on body weight of diabetic rats was presented in Table 2. An average body weight of 110g animals used for the experiment showed fluctuation in body weights after induction of diabetes and treatment.

There was a general increase in body weight of non-diabetic rats administered distilled water. the untreated diabetic rats administered distilled water only showed significant decrease ($p<0.05$) in body weight from days 5 to 10. However, metformin – and extracts – treated

groups showed decrease in body weight on day 5. By day 10, the body weight of the treated rats was normalized and also increased significantly at $p<0.05$. The increased body weight property of the extracts was dose-dependent with best performance shown by 100mg/kg b.w. extract which compared favourably in performance with metformin treated - and non-diabetic groups by day 10.

Table 3: Effects of administration of aqueous extract of *Calotropis procera* leaf on liver malondialdehyde content and antioxidant status in diabetic rats

Group	MDA (units/mg Protein)	Ascorbic acid (Molar)	Reduced glutathione (mg/gtissue)	Catalase (units/g tissue)	Glutathione Peroxidase (units/g tissue)	Superoxide Dismutase (units/g tissue)
Non- diabetic +Distilled Water	4.40 ± 0.10^{a}	3.20 ± 0.10^{a}	42.90 ± 1.10^{a}	55.20 ± 0.20^{a}	67.30 ± 4.70^{a}	52.00 ± 0.70^{a}
Diabetic rats +Distilled Water	6.90 ± 0.00^{b}	1.80 ± 0.10^{d}	27.20 ± 0.00^{c}	41.20 ± 0.10^{c}	55.80 ± 0.00^{d}	34.20 ± 0.10^{d}
Diabetic rats +Metformin	4.60 ± 0.00^{a}	3.20 ± 0.10^{a}	42.00 ± 0.10^{a}	56.50 ± 1.20^{a}	67.10 ± 0.70^{a}	53.00 ± 0.00^{a}
Diabetic rats + 25mg/kg body weight of the extract	5.70 ± 0.10^{c}	2.03 ± 0.01^{c}	34.10 ± 0.10^{c}	42.30 ± 0.40^{e}	59.20 ± 0.10^{e}	41.70 ± 0.30^{c}
Diabetic rats + 50mg/kg body weight of the extract	5.00 ± 0.10^{d}	2.84 ± 0.00^{b}	37.10 ± 0.30^{b}	47.50 ± 0.10^{b}	64.00 ± 0.00^{b}	47.10 ± 1.30^{b}
Diabetic rats + 100mg/kg body weight of the extract	4.60 ± 0.10^{a}	3.21 ± 0.00^{a}	41.80 ± 1.40^{a}	54.10 ± 0.03^{a}	67.70 ± 1.10^{a}	52.40 ± 1.00^{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

Effect of administration of aqueous extract of *Calotropis procera* leaf on malondialdehyde content and antioxidant status of diabetic rats are presented in Table 3. The results revealed significant increase ($p<0.05$) in MDA level of liver in distilled water - treated diabetic rats. The levels of ascorbic acid, reduced glutathione and activities of catalase, superoxide dismutase and glutathione peroxidase decreased significantly ($p<0.05$) in distilled water

treated diabetic rats. Treatment with extract reversed this trend in a manner similar to metformin in the treated animals. There was no significant difference ($p>0.05$) in the values of the biochemical parameters in the diabetic rats treated with 100 mg/kg body weight of the extract when compared with the non-diabetic rats and those administered metformin.

DISCUSSION

The early phase hyperglycemia observed after 1 hour of alloxan administration agreed with the report of Lachin and Reza (2012) on a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment in a similar study. The early phase hyperglycemia occurs for short duration due to alloxan reversal action, permanent diabetic hyperglycemia phase observed within 24-36 hours after administration of alloxan may be attributed to complete degranulation and loss of the integrity of the beta cells (Lenzen, 1998). The permanent diabetic hyperglycemia in the untreated animals must have resulted from complete suppression of the islet response to glucose when high concentrations of glucose were used (Szkudelski *et al.*, 1998). According to Ankur and Shahjad (2012), alloxan action in the pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity.

As shown in Figure 1, the extract mediates decrease in blood glucose through suppression of hepatic gluconeogenesis in the treated groups in this study. In addition to impairment of hepatic gluconeogenesis, the extract may also increase peripheral glucose uptake and insulin sensitivity (Collier *et. al.*, 2006; Fantus and Brosseau, 1986). The correlation between the blood glucose and body weight in terms of decreased blood glucose and increased body weight in the extract treated groups in this study is in accordance with earlier study of Mohammed *et al.*, (2010). The extract possesses anti -wasting syndrome which results from depletion of skeletal proteins through gluconeogenesis and thus slows down or prevents utilization of proteins for energy production.

Malondialdehyde is an index used for the determination of lipid peroxidation (Varshney and Kale, 1990). Overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation (LPO) (Elmegeed *et al.*, 2005). Free radicals especially reactive oxygen species (ROS) has been implicated in a lot of degenerative diseases such as cancer, diabetes, Parkinson and Alzheimer diseases (Oboh and Akindahunsi, 2004). Furthermore, lipid peroxide-mediated tissue damage has been observed in the development of both type 1 and 2 diabetes mellitus and insulin secretion is closely associated

with lipoxygenase-derived peroxides (Kwon *et al.*, 2006). The increased LPO leads to cellular infiltration, Islet cell dysfunction and destruction in diabetes (Kwon *et al.*, 2006).

The increase in MDA level in distilled water - treated diabetic rats may be attributed to increased levels of free radicals caused by hyperglycemia (Materska and Perucka, 2005). Decrease in MDA levels in the extract treated rats suggests the free radical scavenging ability of the aqueous extract of *Calotropis procera* leaves in treated rats.

The present data showing reduction in levels of ascorbic acid, glutathione and activities of antioxidant enzymes in distilled water - treated diabetic rats indicates that alloxan-induced diabetes disrupts the actions of antioxidant status in their liver (Lee *et al.*, 2006). The decrease in SOD activity in distilled water - treated diabetic rats might have resulted from inactivation by H_2O_2 or by glycosylation of the enzyme, which is a common feature in diabetes (Ravi *et al.*, 2004). Decrease in catalase activity could result from inactivation by superoxide radical (Soon and Tan, 2002). Furthermore, catalase is known to be involved in detoxification of high H_2O_2 concentrations, whereas glutathione peroxidase is sensitive to lower concentrations of H_2O_2 (Zhang and Tan, 2000). Reduced activity of glutathione peroxidase in diabetic condition may result from the inactivation of the enzyme from free radicals (Pari and Latha, 2005). According to De Mattia *et al.*, (1998), sustained hyperglycemia that results to tissue damage from prolonged oxidative stress is associated with low cellular levels of the antioxidant glutathione (GSH).

Ascorbic acid (Vitamin C) is a good reducing agent and exhibits its antioxidant activity by donating electron (Oboh and Akindahunsi, 2004). The increased activities of antioxidant enzymes and ascorbic acid levels in extract treated rats explains the free radicals and reactive oxygen species scavenging ability of the extract, portending aqueous extract of *Calotropis procera* leaf as a good dietary source of antioxidants, for the treatment of diabetes and its associated complications. Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by scavenging free radicals produced in the body (Oboh and Rocha, 2007).

CONCLUSION

The aqueous extract of *Calotropis procera* leaf investigated in this study was observed to possess anti-hyperglycemic and antioxidant activities (see Figure 1). The anti-hyperglycemic and antioxidant activities of the extract were also dose-dependent.

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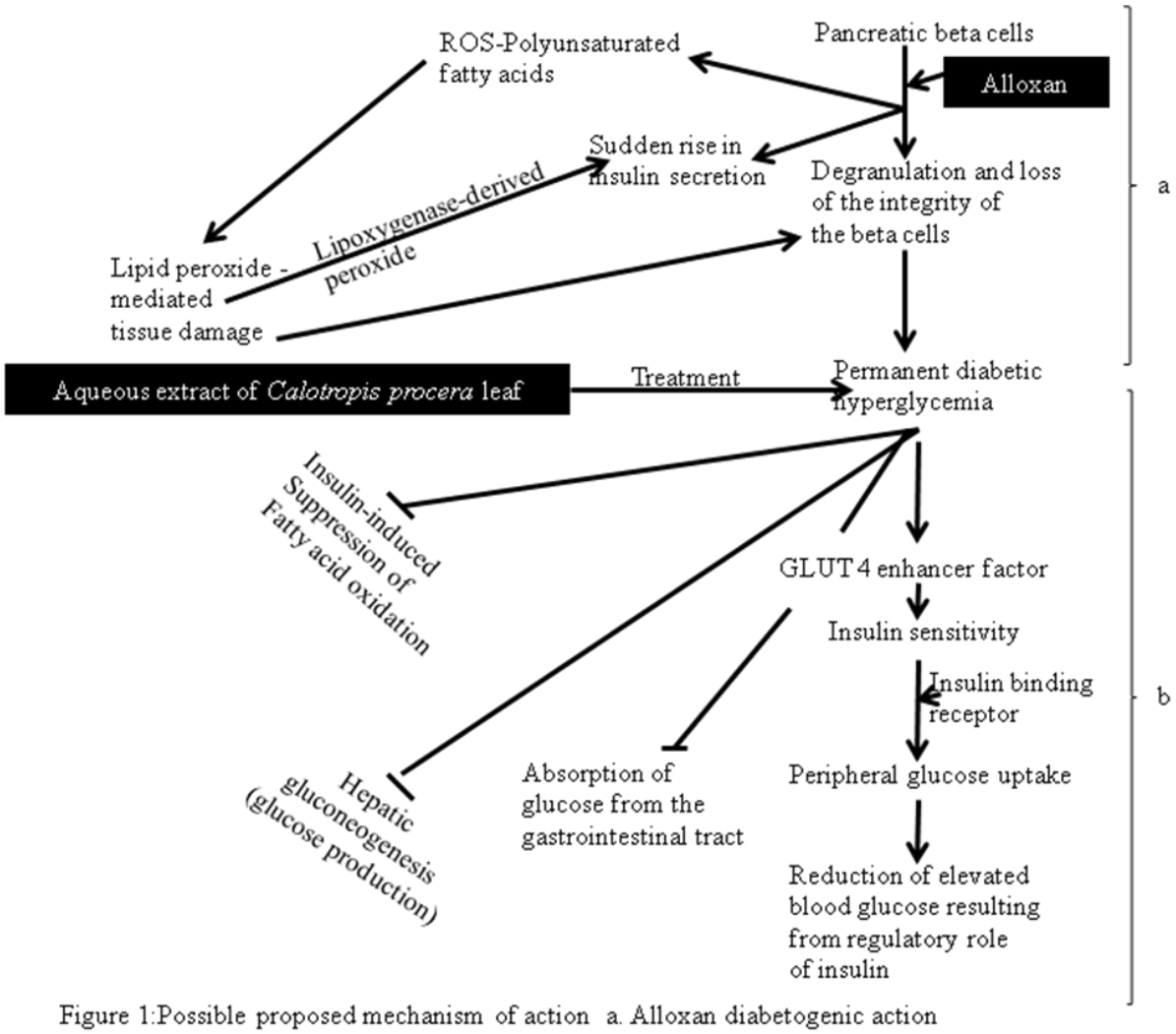

Figure 1:Possible proposed mechanism of action a. Alloxan diabetogenic action
b. Antihyperglycemic and antioxidant actions of *Calotropis procera* leaf extract