The Pharmaceutical and Chemical Journal, 2016, 3(2):161-167

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Evaluation of Anti-hyperlipidemic Potential of Aqueous Extract of *Calotropis procera* Leaf in Alloxan-Induced Diabetic Rat

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Abstract Diabetes affects the heart muscle, causing both systolic and diastolic heart failure; both insulin deficiency and insulin resistance promote dyslipidemia. Effect of aqueous extract of *Calotropis procera* leaf was evaluated on lipid profile and atherogenic markers in alloxan-induced diabetic rat using standard procedures. Adult albino rats (Wistar strain) of mean weight 100.0±2.0g were randomised into six groups (A-F) such that group A (non-diabetic) orally received 0.5ml of distilled 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. The results revealed that blood glucose of the alloxanised rats significantly and progressively reduced in the metformin - and extract - treated animals within 36 hours. Total cholesterol, LDL-c, triacylglycerol, Log (TG/HDL-c), observed in distilled water-treated diabetic rats were significantly reduced ($p<0.05$) in the extract - and metformin - treated rats. The reduction in HDL-c and TG/LDL-c in the distilled water-treated diabetic rats was also reverted back to the range of the non-diabetic controls by the extract and metformin. The significant reduction ($p<0.05$) in the elevated blood glucose level and correction of altered lipid profile observed in aqueous extract treated diabetic animals by *Calotropis procera* leaf to the range of non-diabetic controls in this study indicate the anti-hyperglycemic and anti-hyperlipidemic activity of the extract. In conclusion, *Calotropis procera* leaf can serve as a potent agent for the prevention of coronary heart diseases through its anti-hyperlipidemic property. Further study is recommended for the identification of the bioactive compounds in *Calotropis procera* leaf that exert the anti-hyperlipidemic property offered by the plant.

Keywords Extract, anti-hyperglycemia, anti-hyperlipodemia, CVD

Introduction

Diabetes is a prime risk factor for cardiovascular disease (CVD). Vascular disorders include retinopathy and nephropathy, peripheral vascular disease (PVD), stroke, and coronary artery disease (CAD). Evidence suggests that although hyperglycemia, the hallmark of diabetes, contributes to myocardial damage after ischemic events. Pre-diabetes and the presence of the metabolic syndrome increase the risk of most types of CVD [1-2]. Dyslipidemia and endothelial dysfunction are the mechanisms by which diabetes promote atherosclerosis. The net effect of healthy endothelium is vasodilatory, anti-atherogenic, and anti-inflammatory [3]. In addition, endothelial dysfunction is present, and all of these factors contribute to the increase in atherogenicity, and thus macrovascular disease, found in patients with diabetes [4]. The relationship between elevation of serum lipids and vascular complication of diabetes has long been of interest. The correlation between lipid peroxidation, lipoprotein levels to severity and complication of diabetes mellitus has also been established [5]. Dyslipidaemia is one aspect of cardiovascular complications associated with diabetes mellitus that is under diagnosed and under treated in patients. Diabetes mellitus has impact on lipid metabolism which was substantiated by the fact that all the lipid fractions were elevated in diabetics when compared to healthy controls [5]. Hyperlipidemia is quite common in diabetes; the level of lipid peroxide was raised with the increase in concentration of blood glucose [6]. The increase in lipid peroxidation in the hyperglycemic

condition leads to damage of tissues and organs in diabetic patients [7]. *Calotropis procera* (Sodom apple- English; Bomubomu- Yoruba; Tumfafiya- Hausa; Epuko- Nupe) [8], 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 [9]. In the traditional Indian medicinal system, it has been used for the treatment of leprosy, ulcers, tumors, piles, and diseases of spleen, liver and abdomen [10]. The aqueous extract of the flower has been shown to possess analgesic, antipyretic and anti-inflammatory activities [11]. The objective of this study was to evaluate the anti-hyperlipidemic potential of aqueous extract of *Calotropis procera* leaf through the determination of its effect on serum total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol, TG/ LDL-c and Log (TG/HDL-c in alloxan-induced diabetic rats.

Materials and Methods

Experimental Animals

Thirty adult albino rats – Wistar strain (both sex) of mean weight 100.0±2.0 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, 2015 and were authenticated at the Plant Biology Section of the 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. 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 and Induction of Diabetes

The methods described by Yakubu *et al.*, (2010) were used to prepare the extract and induce diabetes in the animals.

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. Group A was not induced with alloxan while groups B and C were distilled water - 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 for ten days. 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

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 Serum and Tissue Homogenate

The procedures described by Yakubu *et al*., (2010) [12] were used to prepare serum and tissue homogenate. At the end of 10th day, under anaesthesia (diethyl ether, 50 mg / ml), the neck areas of the rats were cleared of fur to expose the jugular veins. The rats were then made to bleed through cut jugular veins into a clean, dry centrifuge tube; the blood was allowed to clot for 30 minutes. The blood samples were centrifuged at 224 x g for 10 minutes using Uniscope laboratory centrifuge (model SM 800B, Suygifriend medicals, Essex, England). Thereafter, the sera were aspirated with Pasteur pipette into clean, dry, sample bottles and kept frozen overnight before being used for the assays. 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 analyses.

Determination of Serum Lipid Profile

Serum total cholesterol, triacylglycerol, HDL-cholesterol and LDL-cholesterol concentrations were determined according to the procedures as described [13-16]. Expressions of Pardo *et al.,* (2008) [17] were used to calculate the molar ratios of atherogenic indices TG/LDL-c and Log (TG/HDL-c).

Results

The effect of administration of aqueous extract of *Calotropis procera* leaf on blood glucose level of diabetic rats is 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.

The effect of administration of aqueous extract of *Calotropis procera* leaf on serum lipid profile of diabetic rats is presented in Table 2. The result revealed significant increase ($p<0.05$) in the serum levels of total cholesterol, LDL-c and triacylglycerol while HDL-c significantly decreased ($p<0.05$) in distilled water - treated diabetic rats when compared to non-diabetic rats. Treatment with extract reverted back the values of the parameters in a manner similar to metformin to non - diabetic controls. There was no significant differences ($p>0.05$) in values obtained for all the parameters in groups treated with 100 mg/kg body weight of extract, metformin and non-diabetic group.

The effect of administration of aqueous extract of *Calotropis procera* leaf on atherogenic indices of diabetic rats is presented in Table 3. The result showed significant decrease in TG/LDL-c and increase in Log (TG/HDL-c) values of distilled water - treated diabetic rats when compared to non-diabetic rats. Treatment with extract reverted the values of atherogenic indices in a manner similar to metformin to non-diabetic control. However, there was no significant differences ($p>0.05$) in the values of atherogenic indices of rats treated with 100 mg/kg body weight of the extract - and metformin - treated rats.

Discussion

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 [18]. Alloxan-induced diabetes has been described as a useful experimental model to study the anti-diabetic activity of several agents [19]. 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 [20]. 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 [19].

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 [21] and revitalisation of the remaining β-cells [22] or inhibiting hepatic gluconeogenesis and by absorption of glucose into the muscle and adipose tissues [23].

Lipids play a vital role in the pathogenesis of diabetes mellitus. Diabetes is associated with profound alterations in the plasma lipid, triacylglycerols and lipoprotein profile resulting in increased risk of coronary heart disease [24]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [25]. In this study, elevated levels of serum lipids such as cholesterol and triacylglycerols were noticed in diabetic rats. Under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes triacylglycerols. Insulin increases uptake of fatty acids into adipose tissue and increases triacylglycerols synthesis as well as inhibits lipolysis. In case of insulin deficiency, lipolysis is not inhibited leading to increased lipolysis which finally results in hyperlipidemia. In diabetic condition, the concentration of serum free fatty acids is elevated as a consequence of free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification cycle is displaced in favour of lipolysis [25].

Insulin deficiency or insulin resistance may also be responsible for the high level of LDL-cholesterol in distilled water - treated diabetic rats in the present study, because insulin has an inhibitory action on HMG-CoA reductase - a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles (Shirwaikar ***et al.,*** 2004). Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, this results in increased production of cholesterol rich LDL particle.

HDL transports cholesterol from peripheral tissues into the liver and acts as a protective factor against coronary heart disease. The level of HDL-cholesterol, which increased after aqueous extract of *Calotropis procera* leaf administration, might be due to increase in the activity of lecithin cholesterol acyl transferase (LCAT), which may contribute to the regulation of blood lipids [26].

The anti-hyperlipidemic effect of aqueous extract of *Calotropis procera* leaves may be due to decreased cholesterol and fatty acid synthesis. Significant lowering of total cholesterol, triacylglycerol, LDL-cholesterol and elevation in HDL-cholesterol by the extract is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions [27]. Therefore, aqueous extract of *Calotropis procera* leaf had significant impact in improving the imbalance in lipid metabolism of diabetic rats [26]. The findings in the present study on the elevation of total cholesterol, triacylglycerol, LDL-cholesterol and decrease in HDL-cholesterol in distilled water - treated diabetic rats agree with earlier reports [12, 26].

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	3.30±0.10^{a}	3.40±0.00^{a}	3.40±0.10^{a}
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 increase and decrease in blood glucose

+= percentage increase in blood glucose

- = percentage decrease in blood glucose

Table 2: Effect of administration of aqueous extracts of *Calotropis procera* leaf on lipid profile of diabetic rats

Group	**Total Cholesterol (mg/ml)**	**HDL-C (mg/ml)**	**LDL-C (mg/ml)**	**Triacylglycerol (mg/ml)**
Non- diabetic+Distilled Water	63.70±0.10^{a}	35.40±2.00^{a}	20.00±4.00^{a}	59.80±2.00^{a}
Diabetic rats +Distilled Water	107.40±0.20^{b}	17.30±1.70^{c}	71.80±6.00^{b}	108. 01±9. 03^{c}
Diabetic rats +Metformin	63.10±0.30^{a}	35.90±1.10^{a}	20.50±0. 05^{a}	59.50±1.50^{a}
Diabetic rats + 25mg/kg body weight of the extract	76.60±0.00^{d}	23.40±1.00^{b}	34.00±2.00^{c}	68.00±0. 04^{b}
Diabetic rats + 50mg/kg body weight of the extract	81.70±3.00^{c}	20.10±0.00^{b}	35.30±0. 01^{c}	70.00±6.00^{b}
Diabetic rats + 100mg/kg body weight of the extract	63.50±3.00^{a}	35.20±1.50^{a}	21.08±0.10^{a}	60.00±0.00^{a}

Values are Means + SEM of 5 determinations

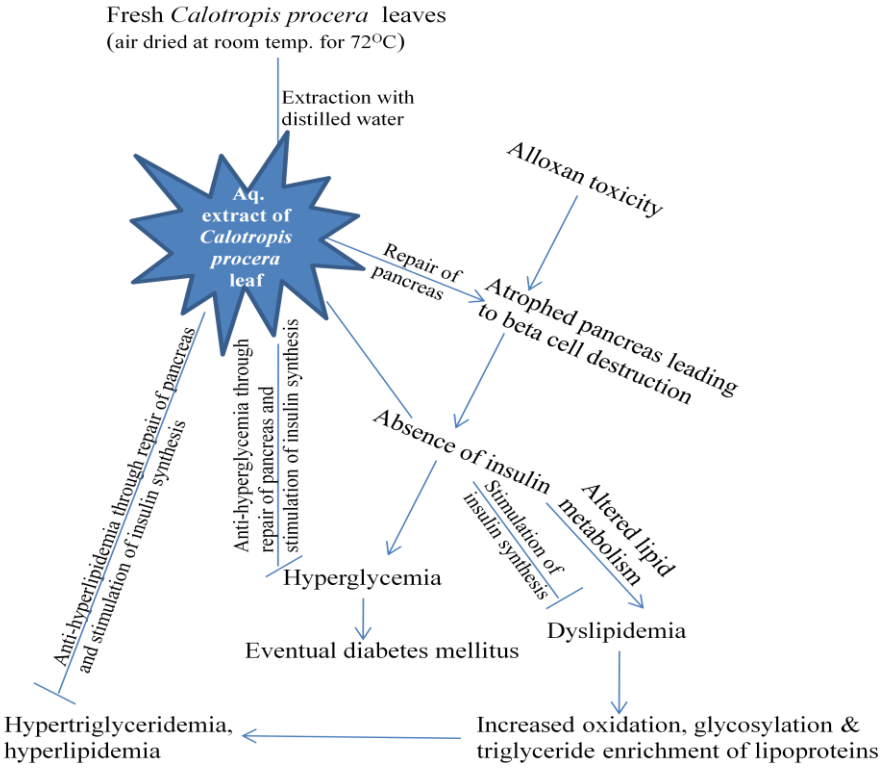
Values down each column carrying different superscript from are significantly different ($p<0.05$) from non-diabetic control

Several lipoprotein-related indices have been linked to cardiovascular diseases in diabetes [28]; these include TC/HDL-c, LDL-c/HDL-c and TG/HDL-c molar ratios, which are adjudged to be good predictive values for future cardiovascular events [29]. However, molar mass ratio log (TG/HDL-c), is also implicated as a significant predictor of cardiovascular diseases [30]. The high value of log (TG/HDL-c) and least value of TG/LDL-c obtained in distilled water - treated diabetic rats which were normalized by the administration of the extract suggest the ability of the extract in correcting disorders of lipid metabolism associated with diabetes mellitus. The high values of atherogenic indices experienced by diabetic rats may be due to the decreased HDL-c values and hypertriglyceridemia which are all due to derangement in lipid metabolism [31-32].

Table 3: Effect of administration of aqueous extract of *Calotropis procera* leaf on atherogenic Indices of diabetic rats

	Atherogenic Indices	
Group	**TG/LDL-C**	**Log (TG/HDL-C)**
Non- diabetic+Distilled Water	2.99±0.07[a]	0.23±0.00[a]
Diabetic rats +Distilled Water	1.50±0.10[c]	0.80±0.01[b]
Diabetic rats +Metformin	2.90±0.01[a]	0.22±0.01[a]
Diabetic rats + 25mg/kg body weight of the extract	2.00±0.00[b]	0.46±0.01[d]
Diabetic rats + 50mg/kg body weight of the extract	1.98±0.02[b]	0.54±0.02[c]
Diabetic rats + 100mg/kg body weight of the extract	2.90±0.01[a]	0.23±0.00[a]

Values are Means + SEM of 5 determinations
Values down each column carrying different superscript are significantly different



Possible mechanism of anti-hyperlipidemic action of aqueous extract of *Calotropis procera* leaf

Conclusion

The present study showed the anti-hyperlipidemic potential of aqueous extract of *Calotropis procera* leaf in alloxan-induced diabetic rat. Therefore, *Calotropis procera* leaf can serve as a potent agent for the prevention, management and correction of coronary heart diseases.

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