

Antidiabetic and hypolipidemic activities of ethanolic leaf extract and fractions of *Carpolobia lutea*

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Abstract

Evaluation of antidiabetic and hypolipidemic activities of ethanolic leaf extract of *Carpolobia lutea* (245,490 and 735 mg/kg b.w.p.o) were carried out in alloxan-induced diabetic albino rats after a single dose (acute study) and after prolonged treatment(chronic study). The activity of the extract was compared with that of referenced drug,glibenclamide (10 mg/kg bw.p.o).The blood glucose level(BGL) was measured by using a glucometer and the various lipids level were estimated using Randox diagnostic kits.Treatment of alloxan diabetic rats with the extract caused a significant ($P < 0.05 - 0.001$) reduction in fasting Blood Glucose levels (BGL) of the diabetic rats both in acute study and prolonged treatment (2 weeks). The activity of the extract was comparable to that of the reference drug, glibenclamide. *C. lutea* treatment showed considerable lowering of serum total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and an increase in HDL cholesterol in the treated diabetic group. These results suggest that the leaf extract of *C. lutea* possesses antidiabetic and hypolipidaemic effect on alloxan-induced diabetic rats which can be exploited in the management of diabetes.

Keywords: *Carpolobia lutea*; Antidiabetic; Hypolipidaemic; Diabetes

Introduction

Carpolobia lutea G. Don (Polygalaceae) is a shrub or small tree up to 5m high. It is widely found in tropical Africa. *C. lutea* is called ikpafum (Ibibio) and cattle stick (English). Ethnobotanically, decoction of the root is used by the Ibibios of Akwa Ibom state of Nigeria as aphrodisiac (Ajibesin *et al.*, 2008; Nwafor and Bassey, 2007) and malarial remedy. Moreover, the leaves are used for the treatment of ulcer and diarrhoea (Nwafor and Bassey, 2007) as well as malarial remedy in some part of Nigeria. The leaves are also used as febrifuge and malarial remedy in Benin (Bero *et al.*,2009). Triterpene saponins have been reported in the leaves of *Carpolobia lutea* (Mitaine-offer *et al.*, 2002) as well as cinnamoyl 1-deoxy glucosides , cinnamic acid and coumaroyl 1-deoxy glucosides (Nwidu et al., 2011). The root can

also be used as anti-inflammatory and anti-arthritic agents (Irvine, 1961; Iwu and Anyanwu, 1982), vermifuge, facilitate childbirth and to treat sterility and headache (Burkill, 1985; Mitai-neoffer *et al.*, 2002). The leaves have been scientifically reported to possess in vitro antiplasmodial (Bero *et al.*, 2009), in vivo antiplasmodial (Okokon *et al.*, 2010), antiulcer and antidiarrhoeal activities (Nwafor and Bassey, 2007; Nwidu and Nwafor, 2009; Nwidu *et al.*, 2011), antimicrobial activity (Etebong and Nwafor, 2009; Nwidu *et al.*, 2012), analgesic (Nwidu *et al.*, 2011; Jackson *et al.*, 2011), contraceptive (Etebong *et al.*, 2011), in vitro antitrypanosomal and antileishmanial activities (Bero *et al.*, 2011). In this study we report the antidiabetic and hypolipidemic activities of the leaf extract of *Carpolobia lutea* in alloxan-induced diabetes in rodents.

Materials and methods

Plant Materials

The plant (leaves) was identified and authenticated by Dr. Magaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo. The leaves were collected from a bush in Nsukara in Uyo Local Government Area of Akwa Ibom State and were authenticated. A voucher specimen of the plant (UUH 998) was deposited in the herbarium of Department of Botany and Ecological Studies, University of Uyo, Uyo.

Extraction

The plant parts (leaves) were washed and shade-dried for two weeks. The dried leaves were further chopped into small pieces and reduced to powder. The powdered leaf was divided into two parts, one part (1.5 kg) was macerated in 97% ethanol for 72 hours to give the crude ethanolic extract while the other part (1.5 kg) was successively and gradiently macerated for 72 hours in each of these solvents; chloroform, ethyl acetate and methanol to give the corresponding fractions of these solvents. The liquid filtrates were concentrated and evaporated to dryness in vacuo at 40°C using rotary evaporator. The yield of each extract was determined; crude ethanolic extract (4.74%), chloroform fraction (0.50%), ethyl acetate fraction (0.30%) and methanolic fraction (1.78%). The dried crude extract/ fractions were stored in a refrigerator at 4°C until use for the proposed experiment.

Phytochemical Screening

Phytochemical screening of the crude leaf extract and fractions was carried out employing standard procedures and tests (Sofowora, 1993; Trease and Evans, 1989), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

Animals

The animals (Swiss albino mice and rats) of both sexes were used for these experiments. They were obtained from University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea Feed) and water *ad libitum*.

Evaluation of antidiabetic and hypolipidemic activities of the Extract and fractions

Induction of diabetes and animal treatment

The animals (male rats) were fasted for 24 hours and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg) in ice cold 0.9% saline (NaCl) solution. The animals were given 2 ml of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia. 72 hours later, rats with blood glucose level (BGL) above 200 mg/dl were considered diabetic and selected for the experiment. The animals were randomly divided into five groups of 6 rats each and treated as follows: Group I: Diabetic rats administered *Carpolobia lutea* extract (245 mg/kg/day) orally for 14 days, Group II: Diabetic rats given *C. lutea* extract (490 mg/kg/day) orally for 14 days; Group III, Diabetic rats administered orally with *C. lutea* extract (735 mg/kg/day) for 14 days. Group IV: Diabetic rats administered orally with chloroform fraction of *C. lutea* (490 mg/kg/day) for 14 days, Group V: Diabetic rats administered orally with ethyl acetate fraction of *C. lutea* (490 mg/kg/day) for 14 days, Group VI: Diabetic rats administered orally with methanol fraction of *C. lutea* (490 mg/kg/day) for 14 days, Group VII: Diabetic rats given Glibenclamide (10 mg/kg/day) for 14 days orally, Group V: Diabetic control rats receiving normal saline (10 ml/kg) for 14 days.

The change in body weight and fasting BGL of all the rats were recorded at regular intervals during the experimental period. For acute study, the BGL was monitored after 1, 3, 5 and 7 h of administration of a single dose of the extract and at the end of 1, 3, 5, 7 and 14 days for prolonged treatments. The BGL was monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded (WHO, 1980).

Evaluation of hypolipidemic activity of the extract and fractions:

After 14 days of treatments with the extract (24 h after the last dose), the rats were anaesthetized with ethyl ether vapour and the blood was collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000 rpm for 15 mins to obtain the sera. Serum cholesterol, triglyceride and High Density Lipoprotein (HDL) levels were measured by enzymatic colorimetric methods using Randox diagnostic kits. All samples were analysed with a wine light Unicam spectrophotometer. The concentrations of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated from the formula of Friedwald *et al.*, (1972). These analyses were done at Department of Chemical Pathology, University of Uyo Teaching Hospital, (UUTH), Uyo.

Results

Phytochemical screening

Phytochemical screening of the crude extract and fractions of *Carpolobia lutea* revealed that the crude extract and methanolic fraction contained tannins, saponins, cardiac glycosides, anthraquinones, flavonoids, steroids and terpenes but in varying quantities. Ethyl acetate

Table 1. Effect of ethanolic root extract of *C. lutea* on body weights of alloxan – induced diabetic rats.

Treatment	Dose (mg/kg)	Day 0	Day 15	% increase/decrease in body weight
Control	0.2ml	84.50±6.72	65.33±5.20	-19.17
Extract	245	89.17±3.19	91.17±2.32	1.00
	490	83.16±6.61	84.49±6.21	1.33
	735	92.83±11.65	96.33±11.76	3.50
Chloroform fraction	490	93.00±9.86	82.50±9.67	-10.50
Ethyl acetate fraction	490	84.17±6.65	85.34±7.43	1.17
Methanol fraction	490	98.83±7.08	101.33±7.12	2.50
Glibenclamide	10	90.00±7.16	93.67±7.00	3.67

Data are expressed as mean ±SEM (n=6).

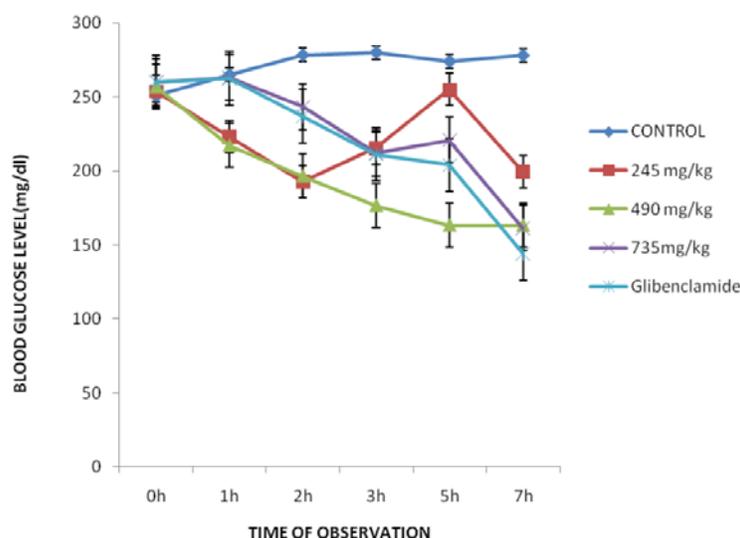


Figure 1. Antidiabetic effect of ethanolic crude leaf extract of *C. lutea* on blood glucose level of alloxan- induced diabetic rats during prolonged treatment.

contained trace of flavonoids and saponins, moderate amount of tannins and a high level of cardiac glycoside, terpenes and steroids. Chloroform fraction contained steroids and cardiac glycosides. Alkaloids and phlobatannins were generally absent.

Effect of leaf extract and fractions of *Carpolobia lutea* on alloxan-induced diabetic rats

There were observable changes in the body weight of treated and untreated alloxan-induced diabetic rats. Treatment of alloxan-induced diabetic rats with ethanolic leaf extract /fractions of *C. lutea* or glibenclamide considerably improved the weights of treated diabetic rats compared to untreated diabetic rats except those treated with chloroform fraction (Table 1).

Acute Studies

The crude extract (245–735 mg/kg) produced dose-dependent reductions in blood glucose level (BGL) alloxan-induced diabetic rats relative to control during acute studies. The-

Table 2: Effect of ethanolic leaf extract and fractions of *Carpolobia lutea* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment.

Treatment	Dose in (mg/kg)	Blood glucose level (mg/dl) in days			
		Day 0	Day 1	Day 7	Day 14
Control	0.2ml	251.0±8.07	294.0±9.01	290.0±10.34	287.0±14.67
Extract	245	254±8.86	190.25±8.98 ^a	176.0±8.78 ^a	159.5±7.34 ^a
	490	257.5±12.97	163.3±5.33 ^a	112.5±4.32 ^a	92.75±3.05 ^a
	735	260.0±12.97	145.9±8.26 ^a	135.25±8.10 ^a	85.5±2.16 ^a
	Chloroform fraction	490	259.5±1.76	282.3±8.38	300.0±11.68
Ethyl acetate fraction	490	254.75±8.05	222.5±18.53 ^a	206.3±8.84 ^a	174.5±5.72 ^a
Methanol fraction	490	256.3±6.77	148.3±9.10 ^a	130.5±6.26 ^a	84.6±5.25 ^a
Glibenclamide	10	260.7±10.74	144.3±10.30 ^a	96.0±10.79 ^a	83.7±7.36 ^a

Data are expressed as mean±SEM. Significant at ^ap<0.001, when compared to control (n=6).

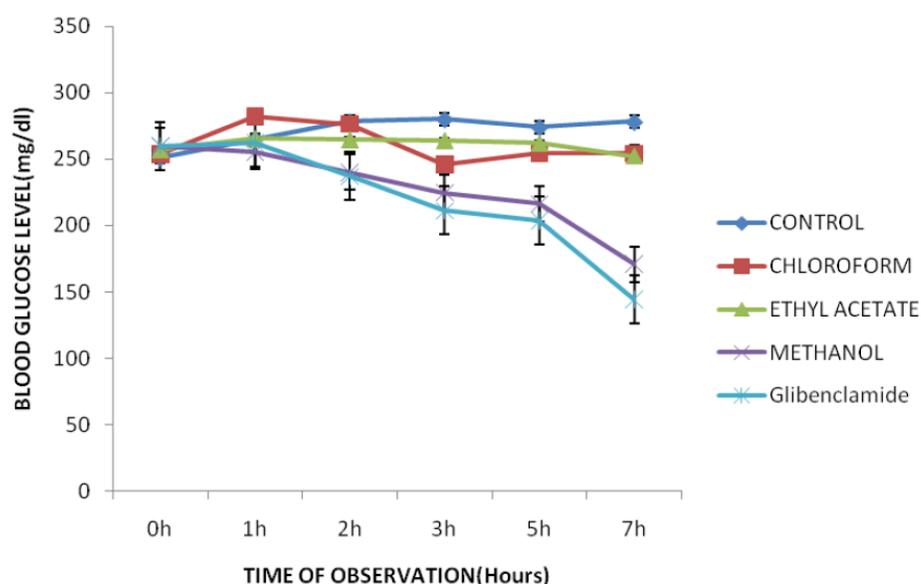


Figure 2: Antidiabetic effect of ethanolic leaf fractions extract of *C. lutea* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment.

se effects were statistically significant ($p < 0.01 - 0.001$) and progressed for 7 hours (Figure 1).

The effect of the highest dose of the extract (745 mg/kg) at the end of 7 hr as well as that of methanol fraction were more significant though less than that of the standard drug, glibenclamide (Figure 2). On prolonged treatment (14 days), the extract produced sustained reductions of BGL in diabetic rats. These reductions were significant ($p < 0.001$) when compared to control (Table 2). The effect of the highest dose of the extract was comparable to that of standard drug, glibenclamide, 10mg/kg, on day 14. The methanol fraction produced the most significant ($p < 0.001$) reduction in blood glucose level of the animals on day 14.

Effect of leaf extract and fractions of *C. lutea* on lipid profile and kidney function of alloxan induced diabetic rats

The ethanolic leaf extract/fractions of *C. lutea* caused decreases in the levels of serum levels of total cholesterol, triacylglycerides, low density lipoprotein (LDL) and very low den-

Table 3: Effect of ethanolic leaf extract and fractions of *Carpolobia lutea* on serum total cholesterol, triglycerides, HDL – cholesterol, LDL – cholesterol and VLDL – chol of alloxan-induced diabetic rats..

Drug	Dose (mg/kg)	Average Serum lipids profile (mMol/L)				
		Total cholesterol	Triglycerides	HDL Cholesterol	LDL Cholesterol	VLDL Cholesterol
Control/Saline	0.2ml	4.08±0.27	2.38±0.15	0.82±0.12	2.18±0.19	1.08±0.07
	245	3.90±0.20 c	2.17±0.14	1.00±0.11	1.74±0.44	0.98±0.06c
Crude extract	490	3.60±0.17 c	1.80±0.22a	1.17±0.14c	1.62±0.09	0.82±0.09c
	735	3.18±0.25 a	1.45±0.31c	1.30±0.11c	1.26±0.06c	0.66±0.15c
Chloroform fraction	490	3.03±0.27 c	1.18±0.28	1.60±0.11c	0.95±0.13	0.49±0.14c
Ethyl acetate fraction	490	2.80±0.24 c	1.08±0.27c	1.55±0.10b	0.76±0.14c	0.49±0.13c
Methanol fraction	490	2.40±0.09c	0.67±0.08a	1.92±0.98c	0.18±0.04c	0.30±0.03c
Glibenclamide	10	2.67±0.18c	1.00±0.18	1.63±0.12c	0.58±0.15c	0.42±0.08c

Data are expressed as mean SEM. Significant at ^ap < 0.05, ^bp < 0.01, ^cp < 0.001. When compared to control (n=6).

sity lipoprotein (VLDL) of extract and glibenclamide treated alloxan-induced diabetic rats. However, the treatment caused elevation in the serum levels of high density lipoprotein (HDL) of the treated diabetic rats. These observed changes were statistically significant (p<0.05-0.001) (Table 3).

Discussion

Evaluation of antidiabetic and hypolipidemic activities of *Carpolobia lutea* leaf extract/fractions were carried out in alloxan- induced diabetic rats. The extract which showed moderate toxicity was observed to demonstrate significant antidiabetic and hypolipidemic activities in alloxan-induced diabetic rats. Some phytochemical compounds such as polysaccharides (Tomoda *et al.*,1985), terpenes and tannins (Reher *et al.*,1991), steroids (Ivorra *et al.*,1989), and alkaloids (Karawya and Wahab,1984) have been implicated in the antidiabetic activities of plants. Phytochemical studies of the leaf extract revealed the presence of terpenes, saponins, tannins and alkaloids. These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. Sulphonylureas cause hypoglycemia by stimulating insulin secretion from the pancreas and these compounds are potent in mild alloxan induced diabetes and inactive in intense alloxan induced diabetes whereby nearly all β -cells have been destroyed (Yallow *et al.*,1960). The observed reduction in BGL of the diabetic rats by glibenclamide in this study portrays an in severe state of diabetes. In this study, continuous treatment with the leaf extract and fractions of for a period of 2 weeks caused significant decrease in BGL of treated rats compared to untreated diabetic rats. This was followed by a significant increase in body weight of the treated rats. Diabetes is characterised by a severe loss in body weight due to loss or degradation of structural proteins (Rajkumar *et al.*,1991). This condition was alleviated by the treatment of the diabetic rats with leaf extract/fractions of *Carpolobia lutea*. Some plants' extracts are reported to exert hypoglycemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin (Pari and Amarnath,2004). While others act through extra pancreatic mechanisms by inhibition of hepatic glucose production (Edduoks *et al.*,2003) or corrections of insulin resistance (Hu *et al.*,2003). These leaf extract and fractions may have acted through one of the above mechanisms. Serum lipids are known to be elevated during severe diabetes and

have been implicated in the development of arteriosclerosis (Minorava *et al.*,2000). The serum lipid levels of the extract treated diabetic rats were significantly reduced after 2 weeks of treatment as against that in the untreated diabetic rats in this study. Diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishnakumar *et al.*,2000). The regression of the diabetic state due to the administration of the root extract may have increased the utilization of glucose, thereby depressing the mobilization of fat. Phytochemical compounds like phenols, tannins, alkaloids, steroids, cardiac glycosides and terpenes present in this extract have been reported to exert antilipidemic activity (Tandon,2005). They may in part be responsible for the hypolipidemic activity of this root extract. In conclusion, the results of this study show that ethanolic leaf extract/fractions of *Carpolobia lutea* possessed antidiabetic properties and also alleviate kidney damage from diabetes. This confirmation justifies its use in ethnomedical medicine for the treatment of diabetes.

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