

Original article:

Hypoglycemic, Antioxidant and Hepatoprotective Activities of Ethanolic Root Bark Extract of *Chrysophyllum albidum* in Alloxan-Induced Diabetic Rats

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Abstract

Objective: The study was aimed at investigating the hypoglycemic, antioxidant and Hepatoprotective effects of *Chrysophyllum albidum* in diabetes induced male Wistar rats. **Methods:** Ethanol root bark extract was administered to thirty rats of six groups A, B, C, D, E and F of five rats each, weighing between 150-170g. Diabetes was induced in Groups B, C, D, E and F using a single intraperitoneal injection of 140mg/kg of Alloxan after an overnight fast. Group A served as the normal control while Group B served as the diabetic control. Group C had metformin of 500mg while Groups D, E and F received 50, 100 and 200mg/kg / bw/ day of the plant extract respectively through orogastric intubation. All the animals were given normal rat chow and water freely. Blood glucose level was determined and the experiment lasted for 3 weeks. On day 21 after an overnight fast, animal were anaesthetized and blood samples were collected by cardiac puncture under inhaled chloroform for the determination of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) assays. The liver function test, ALT, AST and ALP was determined. **Result:** This showed that *C. albidum* and metformin significantly ($p < 0.05$) lowered the Fasting blood glucose (FBS), the activities of SOD and CAT was dose-dependently increase when compared to the diabetic control and there was also a reduction of MDA in the treated groups. There was decrease in the activity of ALT, AST and ALP, which was also dose-dependent. **Conclusion:** The results showed that the plant has significant antidiabetic activity and could therefore be employed for the treatment of diabetes mellitus in which free radicals are implicated.

Keywords: *Chrysophyllum albidum*, alloxan, diabetes mellitus, metformin, pancreas.

Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin¹. It is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial and heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies². This increase has been attributed to increasing afflu-

ence, sedentary lifestyle, increasing obesity, a predominance of hypercaloric diets and an increase in life expectancy³. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated⁴. Till today metformin is the one of the ethical drug approved for the treatment of NIDDM patients. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, there has been increasing

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demand for the use of plant products with antidiabetic activity due to availability, little or no side effects and low cost. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents.

African star apple botanically called *Chrysophyllum albidum* G.Don, belongs to the Sapotaceae family, is commonly found in the Central, Eastern and Western Africa. It is distributed throughout the southern part of Nigeria. In Southwestern Nigeria, the fruit is called "agbalumo" and popularly referred to as "udara" in Southeastern Nigeria. It is a popular tropical fruit tree and widely distributed in the low land rain forest zones and frequently found in villages⁵. It has sweet edible fruits and various ethno-medical uses⁶. Phytochemical profile shows it contains an array of biologically active substances that include alkaloids, tannin, saponin, phenol and flavonoid⁷. Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases⁸. The leaf extract of *C. albidum* can help to thin the blood (antiplatelet effect) as well as regulate the sugar level in blood sugar⁹. The root bark has been known to have antifertility effect on the male¹⁰, also the bark is used as a remedy for yellow fever and malaria while the leaves are used as emollients and for treatment of skin eruption, diarrhea and stomach ache^{11, 12}. However, adequate characterization of hypoglycemic activity has not yet been done on *C. albidum* root bark. This research was performed to characterize the antidiabetic and antioxidant effects of the ethanolic root bark extract of *C. albidum* on alloxanized diabetic rats

Materials and methods

Plant material

The root bark of *Chrysophyllum albidum* was obtained from Ekeakpara Village in Osisioma Ngwa LGA, Abia State in July, 2011. It was authenticated by Pharm. Chief F. N Osuala, Department of Pharmacognosy, Madonna University, Elele. A voucher specimen has been preserved in the laboratory for future reference. The ethanolic extraction process was also carried out in the Pharmacognosy Department of the Faculty of Pharmacy, Madonna University, Elele.

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Preparation of the plant extract

Root bark of *C. albidum* was separated from the root of plant collected; sample was washed in tap water and chopped into bits with a knife on a chopping board. The bits were dried in an uninhabited room for four weeks at room temperature. Dried samples were ground into powder mechanically, using manual grinder. 281.6g of plant powder was soaked in 1.4L of 99% ethanol and kept in refrigerator at 4°C for 48 hours. Mixture was vigorously shaken intermittently for additional 2 hours, to allow for complete extraction. The resulting mixture was rapidly filtered through whatman No 1 filter paper and later with cotton wool to obtain a homogenous filtrate. These filtrates were then concentrated *in vacuo* at low temperature (37- 40°C) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness yielding 67g (8.6%) of brown gummy substance. The extract was later reconstituted in normal saline (0.90% NaCl) at a concentration of 1 g/ml before administration. The extract was refrigerated at 2-8°C until use.

Experimental Animals

Thirty albino rats (males only) of Wistar strain weighing about 150-170g were used in the present investigation. All the rats were given a period of acclimatization for three weeks before the commencement of the experiment. The animals were housed in well ventilated cages and kept under controlled environmental conditions of temperature (25 ± 5°C), relative humidity (50 ± 5°C) and 12 hour light / dark cycle. They were fed *ad libitum* everyday with standard chow diet and were given free access to water.

Induction of Experimental Diabetes

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative¹³. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans¹³. 140 mg of alloxan monohydrate (Sigma chemicals, USA) in

sterile saline per kg body weight of rat was administered intraperitoneally after 48 hour fast (access to only water) to make them more susceptible to developing diabetes. Diabetes was confirmed in alloxan- treated rats with a fasting blood sugar concentration 126mg/dl.

Experimental design

The thirty male Albino Wistar rats were divided into six groups of five rats per group. The crude extract of *C. albidum* and metformin (Hovid Bhd. Malaysia) were dissolved in normal saline (0.9% NaCl) before the treatment which lasted for 21 days.

Group A: Served as normal control and did not receive any treatment.

Group B: Served as diabetic control and received alloxan monohydrate and normal saline

Group C: Alloxan monohydrate + Metformin (500 mg/kg/bw) and served as standard

Group D: Alloxan monohydrate + Ethanolic extract (50 mg/kg/bw)

Group E: Alloxan monohydrate + Ethanolic extract (100 mg/kg/bw)

Group F: Alloxan monohydrate + Ethanolic extract (200 mg/kg/bw)

Estimations

Fasting blood glucose was determined after depriving food overnight with free access of drinking water. Blood glucose was estimated using Glucometer and test strips (Prestige, U.S.A) set at code 21, with blood obtained from the tail vein of the rats using syringe and needle. Blood glucose levels were estimated after 1hr, 2hrs, 14th and 21st day of the treatment.

Effect of extract on average body weight of rats
Initial, 3rd, 9th, 16th and 20th days respectively, the rat weights were taken and the difference in weight from the initial weight per group was calculated.

Blood collection and preparation of sample

At the end of the treatment period, the rats were anaesthetized in chloroform prior to dissection. The blood was then collected by cardiac puncture into lithium heparinized bottles. Blood was centrifuged at 10, 000 rpm for 15 minutes into clean bottles and stored at -20°C until required for bio-

chemical assays

Analysis of biochemical parameters

Serum MDA was measured by a thiobarbituric acid assay procedure (14), which was calibrated using 1,1,3,3, - tetraethoxypropane (Sigma Chemicals, St. Louis, MO, USA) as a standard. Results were expressed as nanomoles of MDA per millimeter of serum. Superoxide dismutase (SOD) was assayed utilizing the method described by Sun and Zigma¹⁵ and serum catalase (CAT) activity was determined according to the method of Beers and Sizer as described by¹⁶. ALT activity and AST activity were determined; using commercially available kits (Randox Laboratories Ltd., UK).while ALP was assayed using Diagnosticum Zrt. Diagnostic kit (Diagnosticum Zrt., Budapest, Hungary).

General Protocol

Cage- side examination were conducted daily to detect signs of toxicity (loss of hair, behavioral abnormalities, dead rats, salivation, refusal of feed, weight loss and chew jaw movement). All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the care and use of Animals and approved by the Department Committee on the Use and Care of Animals.

Statistical analysis

All biochemical results were expressed as Mean±SEM significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by student's t-test using the SPSS statistical analysis program. Statistical significance was considered at $p < 0.05$.

Results

Body weight

There was a decrease in the body weight of diabetic control and treated groups when compared to the control animals that gained weight. Alloxan mediated body weight reduction was statistically significantly by the ethanolic root bark extract in dose dependent fashion (at 50, 100 and 200 mg/kg body wt). Results are shown in Table I.

Table I: Effect of Ethanolic Root Bark Extract of *C. albidum* on body weight of rats (Mean \pm SEM).

Groups	Initial	Day3	Day 9	Day 16	Day 20
A	167 \pm 4.51	167 \pm 6.50	170 \pm 5.60	183 \pm 1.00	186 \pm 4.25
B	170 \pm 5.77	156 \pm 6.50	147 \pm 2.33	130 \pm 1.81	121 \pm 1.31
C	166 \pm 3.33	151 \pm 7.76	143 \pm 6.24	136 \pm 3.84	134 \pm 5.78*
D	154 \pm 3.33	146 \pm 1.47	135 \pm 7.7	132 \pm 6.88	130 \pm 7.68*
E	156 \pm 3.33	148 \pm 3.84	138 \pm 5.66	133 \pm 7.21	130 \pm 0.02*
F	150 \pm 3.33	142 \pm 1.13	137 \pm 4.17	130 \pm 2.49	112 \pm 4.33*

Values are Mean \pm SEM; n=5. *P < 0.05, compared to diabetic control

Fasting blood glucose level

In this study, increase in blood glucose level was observed on induction of diabetes mellitus in the rats' models. Treatment of diabetic rats with ethanolic root bark extract of *C. albidum* (50, 100, 200mg/ kg body weight/ day) and metformin (500

mg kg⁻¹) for 21 days after establishment of hyperglycemia resulted in significant reduction (P<0.05) of fasting blood glucose levels. However, metformin caused a greater hypoglycemic effect than *C. albidum* ethanolic root bark extract. The results are shown in Table II.

Table II: Effects of *C. albidum* on fasting blood glucose levels of alloxan-induced diabetic rats. Values given represent in Mean \pm SEM.

Groups	Before	1 day	1 hour	2 hours	14days	21 days
A	Alloxan admin. (mg/dl)		Post extract administration (mg/dl)			
B	48 \pm 3.80	50 \pm 2.72	50 \pm 1.73	54 \pm 2.50	58 \pm 17.0	62 \pm 3.44
C	47 \pm 0.50	233 \pm 71.0	158 \pm 19.50	288 \pm 14.0	189 \pm 4.0	131 \pm 8.50
D	53 \pm 11.0	204 \pm 72.50	151 \pm 41.0	108 \pm 25.0	45 \pm 2.50	34 \pm 2.50*
E	38 \pm 3.50	146 \pm 14.0	109 \pm 13.43	73 \pm 5.0	60 \pm 2.00	29 \pm 1.00*
F	48 \pm 5.00	147 \pm 12.50	129 \pm 27.57	92 \pm 8.00	58 \pm 17.0	50 \pm 2.00*
	51 \pm 3.00	133 \pm 1.50	130 \pm 4.50	124 \pm 5.00	61 \pm 3.00	46 \pm 1.00*

Values are MEAN \pm SEM; n=5; *P < 0.05, compared to diabetic control

In vivo antioxidant activity

Lipid peroxidation (MDA) were significantly (P<0.05) higher in the diabetic (5.86 \pm 0.03) than the control group (3.21 \pm 0.12). The treatment with ethanolic root bark extracts of *C. albidum*; 50mg/kg body wt (1.90 \pm 0.02), 100mg/kg body wt (2.00 \pm 0.003) and 200mg/kg body wt (2.19 \pm 0.05)

respectively, showing evident of dose dependent decrease when compared to the diabetic control. The metformin- treated diabetes also shows significant low level (2.11 \pm 0.05). Beside the activities of SOD and CAT in all treated groups were significantly (P<0.05) increased compared to the diabetes control (Table III).

Table III: Effect of ethanolic root bark extract of *C. albidum* on antioxidants and lipid peroxidation

GROUPS	SOD (U/ml)	CAT (U/ml)	MDA (μ mol/ml)
A	50.32 \pm 0.18	18.10 \pm 0.05	3.21 \pm 0.12
B	30.90 \pm 0.05	10.22 \pm 0.09	5.86 \pm 0.03
C	45.00 \pm 0.25	16.34 \pm 0.02	2.11 \pm 0.05*
D	40.12 \pm 0.06	11.16 \pm 0.003	1.90 \pm 0.02*
E	36.12 \pm 0.05	11.00 \pm 0.17	2.00 \pm 0.003*
F	35.08 \pm 0.04	10.88 \pm 0.005	2.19 \pm 0.05*

Values are MEAN \pm SEM; n=5; *P < 0.05, compared to diabetic control; SOD, superoxide dismutase; CAT, catalase; MDA, malonaldehyde

Liver function enzymes

As shown in table iv, oral administration for 20 days of *C. albidum* and metformin decrease the activities of the serum levels of ALT, AST, and

ALP. However, the attenuating effects of *C. albidum* and metformin groups were significantly lower (P<0.05) than that of the alloxan induced diabetes.

Table IV: Effect of Ethanolic Root Bark Extract of *C. albidum* on liver function test (Mean ± SEM)

Groups	ALT	AST	ALP
A	35 ± 0.61	25 ± 0.21	38 ± 0.05
B	6 ± 0.32	16 ± 0.14	23 ± 0.04
C	5 ± 0.10*	12 ± 0.01*	17 ± 0.05*
D	2 ± 0.06*	12 ± 0.15*	17 ± 0.01*
E	2 ± 0.03*	5 ± 0.03*	8 ± 0.07*
F	1 ± 0.05*	2 ± 0.28*	7 ± 0.05*

Values are Mean ± SEM; n=5. *P < 0.05, compared to diabetic control

Discussion

The treatment strategies of diabetes mellitus include nutritional therapy, insulin injection, treatments with the various classes of oral hypoglycemic agents which could be synthetic or of herbal origin¹⁷ and/or combination of any of these strategies. However, in the African herbal management of DM, varieties of medicinal plants are employed, some of which have been widely investigated and reported¹⁸. Researchers have accordingly reported significant weight reductions in untreated diabetic rat models^{19, 20}. This was also the observation in this work with untreated alloxan-diabetic rats. The gradual reduction in weight of untreated diabetic rats over the 21 days clearly indicates that the deterioration in the glucose control mechanism would probably climax in the death of the animal if untreated. The result of the effect of the extract on the average weight pattern in the treated rats shows a pattern of weight lost with progressive increase dose of the extract when compared with untreated control rats. This extract can therefore be used not only to control glucose homeostasis in diabetes but to control obesity alike.

Oxidative stress is a confounding factor in diabetes mellitus and it contributes to the pathogenesis²¹ and complications²² of this disease. In alloxan-induced diabetes has been shown to induce free radical production and causes cellular injury, also it increases pancreatic oxidative stress which are associated with the progression of this disease²³ as was also obtained in this study.

Treatment of the rats with *C. albidum* extract and metformin exhibited antioxidant effect by increasing CAT and SOD activities, and decreasing MDA

levels, suggesting that antioxidant enzymes had been involved in this repair process of membrane damage. MDA is the end product of lipid peroxidation and measures free radical generation. Thus, validating the scavenging property of the extract against free radicals generated.

Majority of plants have been reported to exert their hypoglycemic actions via the presence of insulin-like phytochemicals²⁴. It is however evident from this research that *C. albidum* extracts studied contains hypoglycaemic agents capable of lowering blood glucose level in alloxan induced diabetic rats when administered. A marked normalization of blood glucose levels in these animals was achieved after 3 weeks of treatment. Therefore, the effectiveness of the extract depends, probably on the accumulative effect of active properties, including terpenoids that are known to reduce glycaemia through many mechanisms which include insulin-like activity, inhibition of gluconeogenesis and glycogenolysis²⁵. Other possible mechanism includes the stimulation of cells and subsequent release of insulin and activation of insulin receptors.

It has also been documented that flavonoids, tannins and phenolic content of therapeutic plants contributes immensely to antioxidant activity of the plants, which may be responsible for protecting the cells against the oxidative stress, possibly by increasing the endogenous defensive capacity of the pancreas to combat oxidative stress induced by alloxan. These constituents of this plant may have stopped further destruction of the remaining beta cells in the islet by mopping up the circulating reactive oxygen species generated by the alloxan to destroy the beta cells and then allowing other phytochemicals of the plant to induce regenerative activities²⁶⁻²⁸.

It is well documented in the literature that an increased serum or plasma level of ALT is specific for and predictive of hepatocellular damage^{29, 30}. Other liver enzymes markers such as AST and ALP are known to be elevated to significant levels in liver diseases³¹. In the present study, these biochemical alteration were significantly (p > 0.05) attenuated by the oral administration of ethanolic extract of *C. albidum* and metformin for 21 days.

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The decrease in the liver function test; ALT, AST and ALP indicates the reflective ameliorating effects extract of *C. albidum* and metformin on serum liver enzymes.

Conclusion

The results shows that the ethanolic root bark extract of *Chrysophyllum albidum*, through its activities, increases the activities of SOD and CAT; thereby reducing MDA level, and thus decreased glycaemia. Furthermore, decreasing

lipid peroxidation in the pancreas of diabetic rats exposed to *C. albidum* suggests the beneficial potential of the herb in the amelioration of ROS-associated with diabetes mellitus. The decrease in the liver function test indicates that the extract of *C. albidum* has hepatoprotective effect on the liver.

Conflict of no interest statement

We declare that we have no conflict of interest.

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