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Antioxidant, hypolipidemic and Hypoglycemic Effect of Ethanol Leaf Extract of *Chrysophyllum albidum* on Streptozotocin- Induced Diabetic Rats.

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ABSTRACT

The purpose of the present study was to investigate the antioxidant and anti-diabetic effect of *Chrysophyllum albidum* in streptozotocin-induced diabetic rats. Fifty Sprague-Dawley rats (170-200g) were used, of which twenty rats were randomised into four groups (n= 5). Group A received normal saline 10 ml. Group B, C and D received *C. albidum* by gavage at a dose of 125, 250 and 500 mg/kg respectively. The prophylactic study was performed with thirty rats divided into six groups (n=5). Groups E and F received only normal saline; group G received 2 mg/kg of glibenclamide; groups H, I and J received 125, 250, and 500 mg/kg/day of ethanolic leave extracts of *C. albidum* respectively. With the exception of group E, animals in groups F-J was challenged with 50 mg/kg of streptozotocin intraperitoneally after two weeks of oral administration of normal saline, extract and glibenclamide. Administration of normal saline, extract and glibenclamide was continued for another two weeks. The animals were fasted overnight before they were sacrifice on day 14th and their blood samples and pancreas were collected for biochemical assays. Results showed significant elevation in Superoxide dismutase, Catalase, and Glutathione activities ($p< 0.05$); and decrease in malondialdehyde. The extract significantly increased ($p < 0.05$) High density lipoprotein and also significantly reduced ($p < 0.05$) Low density lipoprotein, Triglycerides and Total cholesterol. It can be concluded that *C. albidum* possesses hypoglycemic, antioxidant and hypolipidemic potentials, thereby justifying support for its anti-diabetic use in folk medicine.

Keywords: *Chrysophyllum albidum*, Diabetes, Streptozotocin, Oxidatives stress, lipid profile

INTRODUCTION

Diabetes mellitus is a metabolic disorder that is marked by elevated blood glucose concentration and excretion of glucose in urine[1]. It is usually associated with oxidative stress, accompanied by excessive production of free radicals[2]. Elevated generation of ROS and the simultaneous decline in antioxidative defense mechanisms observed in diabetic patients could promote the development of complications associated with diabetes mellitus [3], such complications include cardiovascular disorders, blindness, renal failure, neuropathies, and cancers [4,5].

Abnormal lipid metabolism causes deranged levels of Total Cholesterol, Triglycerides, Low density lipoprotein, High density lipoprotein, which is one of the reasons for premature atherosclerosis in patients with diabetes mellitus [6]. The non-insulin-dependent diabetic (NIDDM) patient with mild fasting hyperglycemia commonly has mild hypertriglyceridemia due to overproduction of TG-rich lipoproteins in the liver, associated with decreased high-density lipoprotein levels [7]. High levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease and stroke [8].

Many herbal products have been prescribed for the management of diabetes mellitus in ancient and recent literature [9]. Available literatures show that there are more than 400 plant species showing anti-diabetic activity with the possible use in the treatment of DM and its complications [10]. Several plant extracts have been examined for their antidiabetic properties in an attempt to recognize alternative treatment strategies that pose less of a hazard for diabetics [11].

Chrysophyllum albidum. (Family: Sapotaceae) has many common names including white star apple agbalumo (Yoruba), udara (Igbo) and agwaluma (Hausa). It is a popular tropical fruits widely distributed in Nigeria. The plant *C. albidum* have been employed in folk medicine for the treatment of diseases. The seeds from the cotyledons are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria and also possess anti-hyperglycemic and hypolipidemic effects [12]. Hypoglycemic, Antioxidant and Hepatoprotective activities of the root bark has been reported [13]. The root bark has been known to have antifertility effect [14], 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, diarrhoea and stomach ache [15, 16] and also has antiplatelet effect [17]. The fruit pulp is rich in vitamin C and iron and an excellent source of raw material for industries [15, 18]. The purpose of this study is to evaluate the effects of *C. albidum* on the hypoglycemic, oxidative stress markers and lipid profiles on STZ-induced diabetes using Sprague-Dawley rats.

MATERIALS AND METHODS

Plant material

The fresh leaves of *C. albidum* were collected on September, 2015 in Elele of Rivers State, and authenticated by Mr O. O. Oyebanji, a taxonomist in the Department of Botany, University of Lagos, Nigeria. It was assigned a voucher number LUH 7458 and was deposited in the herbarium for future reference.

Plant extraction

C. albidum leaves were air dried for two weeks and then blended into powder. The method used was as described by [19]. The powdered leaves of *C. albidum* two kilograms were soaked in 17 L of ethanol in a glass jar for four days at room temperature, after which the extract was filtered using a What man no. 1 filter paper and cotton wool. It was concentrated at 50°C using a rotary evaporator and further concentrated using water bath at 48°C. The yield of the ethanol extract was found to be 168 g giving a percentage yield of 21.6 %. The extract was stored in a beaker covered with aluminium foil and stored in a refrigerator at 4°C until when needed.

Experimental Animals

Fifty adult Sprague-Dawley rats of both sexes (170-200g) were obtained from the breeding colony of the National Institute of Medical Research (NIMR) Yaba, Lagos. They were housed in cages at room temperature under standard conditions and maintained in a 12h light/dark cycle. The animals were fed on pelletized growers feed and water *ad libitum*. The animals were allowed to acclimatize for two weeks in the animal house of the Department of Anatomy, University of Lagos, Nigeria before commencement of the experiment.

Experimental design and treatments

A (Hypoglycaemic study)

Study on normoglycemic animals

Four groups of five rats each were fasted overnight. Group A received distilled water 10 ml. Group B, C and D received the *C. albidum* that is administered by gavage at a dose of 125, 250 and 500 mg/kg body weight respectively. Blood samples were drawn by puncture from the tail immediately at 0, 4hr, 8hr and 12 hrs to check for blood glucose levels [20,21].

Experiment B (Prophylactic study)

Induction of experimental diabetes

Experimental diabetes mellitus was induced by a single intra-peritoneal injection of a freshly prepared streptozotocin (STZ) solution (Sigma, St. Louis, MO, USA) (50 mg/kg in cold citrate buffer 0.1 M, pH 4.5) to overnight fasted rats. Seventy two (72) hours after STZ injection, blood was taken from tail vein of the rats. Animals having blood glucose levels $\geqslant$ 300mg/dl were considered diabetic and included in the study.

Animals grouping and Administration of *C. albidum*

The prophylactic study was performed with 30 rats of both sexes randomly divided into 6 groups (E-J) of five rats per group: groups E and F received only normal saline; group G received 2 mg/kg of glibenclamide; groups H, I and J received 125, 250, and 500 mg/kg/day of the leave extract respectively. With the exception of group E, animals in groups F-J was challenged with 50 mg/kg of streptozotocin intraperitoneally after two weeks of oral administration of normal saline, extract and the dosage of glibenclamide. The animals were considered as being diabetic if the blood glucose values were $\geqslant$ 300mg/dl 72 hours after STZ injection.

Treatment was continued for another two weeks , blood glucose levels were measured every three days during the experimental period after which the animals were fasted overnight, anaesthetized under chloroform fumes, sacrificed and their sera collected for biochemical assay.

Biochemical analysis

Blood Glucose Level (BGL) was measured with Accu-check (R) Active glucose strips in Accu-check Active (R) (Roche Diagnostic, Mannheim, Germany) test meter and expressed as mg/dl, using blood obtained from the tail vein of overnight fasted rats

Antioxidant Enzymes Assay

Superoxide Dismutase (SOD) activity was determined by method described by [22]. Catalase activity was determined according to[23]. The reduced glutathione (GSH) content was estimated according to the method described by [24]. Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of [25].

Plasma Lipid Profile Determination

Plasma Total cholesterol (TC) levels, Plasma Triglyceride (TG), High Density Lipoprotein (HDL) were determined using a Randox diagnostic kit [26,27]. Low Density Lipoprotein (LDL) was calculated using the empirical equation of[28].

Estimation of serum insulin level

The estimation of serum insulin levels was done by radio-immunoassay (RIA) using Mercodia Ultrasensitive Rat Insulin (INS) ELISA Kit (Catalog. No: WAR-617).

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments were examined and approved by the College of Medicine University

of Lagos Health Research Ethics Committee and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed using GRAPH PAD Prism software package, Version 5.0. All the data were expressed as mean ± standard deviation (SD). The comparison within groups was evaluated utilizing independent Turkey T test and one way analysis of variance (ANOVA). The value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Hypoglycaemic effect of *C. albidum* on normal rats

In the hypoglycaemic study as shown in table 1, there was appreciable decrease in glycaemic level in groups C and D (47.6±6.28 and 46.6±10.54) when compared to the control group A (51.6±6.89). However, at group B, significant ($p< 0.05$) decrease occurs after 4 hour of the oral administration of the extract (48.2±8.35) also, the hypoglycaemic effect produced at 12 hour was more (33.6±8.87).

Table 1: Hypoglycaemic assessments of *C. albidum* leave extract

Group	0 hr	4hr	8hr	12hr
A	71.8± 8.13	61.2±7.86	56.4±6.05	51.6±6.89
B	72.4±9.75	48.2±8.35*	42.6±7.92 *	33.6±8.87*
C	70.0±3.35	56.4±4.41	51.8±6.11	47.6±6.28
D	74.8±5.85	63.8±13.41	55.4±14.15	46.6±10.54

Values are expressed as mean ± SD of n= 5 per group.*represents significant difference when compared with A ($p< 0.05$)

Key: A= Control, B= *C. albidum* 125 mg/kg, C= *C. albidum* 250 mg/kg, D= *C. albidum* 500 mg/kg

Pancreatic anti-oxidant status of *C. albidum* leaf activity

The table 2 showed the activities of antioxidant enzymes and malondialdehyde (MDA). There was a statistically significant ($p< 0.05$) decrease in the diabetic untreated group F in reduced glutathione (GSH), superoxide dismutase(SOD) and Catalase (CAT) activities and increase in the MDA content when compared to group E (control). The GSH and CAT activities and MDA content in group G (glibenclamide) and the treated groups(H, I and J) showed no significant difference, however, there was appreciable increase of SOD and CAT at 125mg/kg (group H) when compared to the control (group E). There was a statistical significant ($p< 0.05$) increase in GSH, SOD and CAT activities in glibenclamide and treated groups with appreciable increase in CAT at a dose of 125 mg/kg group H, however with significant ($p< 0.05$) decrease in MDA when compared to the diabetic untreated group F.

Table 2: Effect of C. albidum on pancreatic oxidative stress parameters

GROUP	DOSE mg/kg Bw/Day	GSH µmol/ml/mg Protein	SOD µmol/ml/min/mg Protein	CAT µmol/ml/min/mg Protein	MDA µmol/ml/mg Protein
E	10 ml	0.82±0.24	2.80±0.03	20.24± 0.17	0.06 ± 0.03
F	10 ml	0.49±0.01*	1.44±0.01*	7.63±0.80*	1.40±0.00*
G	2	0.81±0.05^	2.45±0.17*^	18.35±0.14^	0.05±0.03^
H	125	0.76±0.01^	1.70±0.08*^	11.45±0.06*	0.06 ±0.01 ^
I	250	0.76±0.01^	2.55±0.18*^	15.42±0.19^	0.04±0.01^
J	500	0.75±0.01^	1.73±0.01*^	13.38±0.22^	0.05±0.00^

Values are expressed as mean± SD. N= 5 in each group. *= significant difference when compared with E ($p<0.05$). ^= significant difference when compared with F ($p<0.05$)

KEY: E=control- Distilled water, F= Diabetic control (normal saline), G= 2mg/kg Glibenclamide, H= 125 mg/kg *C. albidum*, I= 250 mg/kg *C. albidum*, J= 500 mg/kg *C. albidum*

GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde

Effect of *C. albidum* on serum lipid profile

Serum levels of total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-c) was increased significantly ($p<0.05$) in Diabetic Control (group F) in respect to the control (group E). The treated groups (G, H, I and J) showed significant recovery ($p<0.05$) when compared to the control group E, except the *C. albidum* at a dose of 500 mg/kg (group J) for CHOL and TG at 125 mg/kg (group H) which were significantly ($p<0.05$) increased and decreased respectively when compared to the control group E. This study also showed serum high density lipoprotein cholesterol (HDL-c) concentration level was decreased significantly ($p<0.05$) as a result of the effect of diabetes, which was severe in the Diabetic Control group when compared to the control (group E). The treated groups showed significant recovery ($p<0.05$) when compared to the Diabetic Control (group F)

Table 3: Effect of treatment on serum lipid profile

GROUPS	DOSE mg/kg bw/day	TC nmol/L	TG nmol/L	HDL-C nmol/L	LDL-C nmol/L
E	10 ml	0.71 ±0.04	0.64± 0.01	0.81±0.09	0.18±0.02
F	10 ml	2.53±0.07*	1.29±0.08*	0.42±0.05*	1.08±0.06*
G	2	0.66±0.07^	0.64± 0.00^	0.74±0.09 ^	0.18±0.06^
H	125	0.75±0.03^	0.50±0.04*^	0.71±0.01^	0.18±0.01^
I	250	0.72±0.02^	0.61±0.03^	0.86 ± 0.03^	0.21±0.03^
J	500	0.88±0.04* ^	0.63±0.01^	0.82±0.03^	0.23±0.01^

Values are expressed as mean± SD. N= 5 in each group.*= significant difference when compared with E ($p<0.05$). ^= significant difference when compared with F ($p<0.05$)

CHOL: Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein, LDL-C: Low density lipoprotein

Effects of *C. albidum* on serum Insulin level

To assess insulin secretion in these animals, fasting insulin levels (Table 4; at 0 and 30 min) in diabetic untreated group was significantly decreased ($p<0.05$) when compared to the control. However, there was a significant ($p<0.05$) positive impact on insulin levels in the glibenclamide (2 mg/kg) and treated groups (H, I and J) when compared to the diabetic untreated group F. When plasma insulin levels were measured 30 min after a glucose challenge in rats, it was found that circulating insulin levels were significantly reduced in the diabetic untreated. However, treatment with glibenclamide and *C. albidum* resulted in a significant ($p<0.05$) increase in insulin level when compared to the diabetic untreated.

Table 4: Effect of *C. albidum* on insulin level

Groups	0 min	30 mins
E	13.63 ±0.72	14.31±0.58
F	3.29±0.49*	2.80±0.52*
G	6.47±0.50*^	7.45±0.57*^
H	5.54±0.62*^	6.32±0.74*^
I	6.05±0.64 *^	7.40±0.73*^
J	6.14±0.64*^	7.74±0.62*^

Values are expressed as mean± SD. N= 5 in each group. *= significant difference when compared with E ($p<0.05$). ^= significant difference when compared with F ($p<0.05$)

DISCUSSION

In the hypoglycaemic study, the activities of *C. albidum* were evaluated with normal rats. At doses of 250 and 500 mg/kg, the leaf showed appreciable hypoglycaemic effects. But at 125 mg/kg, a significant ($p<0.05$) decrease in glycaemia was observed. It has been observed that hypoglycaemic activities occur from the stimulation of pancreatic beta cells to release insulin [29]. [30] reported the phytoche-micals, alkaloids, flavonoids, tannins and glycosides to have hypoglycaemic effects in normoglycaemic rats. Glycosides are implicated with strong hypoglycaemic activities [31]. It is therefore presumed that these active components present in the extract were largely responsible for the hypoglycaemic effects.

Oxidative stress has been suggested as mechanism underlying diabetes mellitus and diabetic complications[32]. Hyperglycemia in diabetes mellitus causes a depletion of the cellular antioxidant defences and increases the levels of free radicals [33]. Abnormal elevated levels of free radicals and the simultaneous reduction of antioxidant defense can result in damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance [34]. These enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to STZ [35]. Superoxide dismutase (SOD), a metallo protein is the most sensitive and most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical [36]. While Catalase (CAT) is an enzymatic antioxidant widely distributed in all tissues. It is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 And OH. Therefore, the reduction in the activity of catalase may result in a number of deleterious effects due to accumulation of hydrogen peroxide [37]. The pancreas has a relatively weak intrinsic defense system against oxidative stress [38], hence the need to protect it from oxidative stress.

In this study, the pancreatic oxidative status, shows the decreased activity of SOD, CAT and GSH of diabetic animals, in addition the increase in malondialdehyde (MDA) levels in pancreas suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ethanol extract of *C. albidum* significantly increases the antioxidant enzymes and decrease in MDA was observed. These findings suggest that the *C. albidum* may exert antioxidant activity and protect the tissue from lipid peroxidation.

Abnormalities of lipid profile are one of the most common metabolic complications of diabetes mellitus which is found in about 40% of diabetes [39]. Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoprotein plays a significant role in the proatherogenesis of vascular complications in diabetes mellitus [40]. Insulin is a potent inhibitor of the lipolysis [41], the lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue [40]. The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride [42]. The increased free fatty acid concentration promotes the production of more acetyl co A and cholesterol [41]. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots [43].

When much LDL-cholesterol circulates in the blood, it can slowly build up in the inner walls of the arteries that feed the heart and brain. It can also form plaque that narrows the arteries, a condition known as arthrosclerosis. An elevated level of triglyceride is a consistent feature in diabetic dyslipidemia [44]. Hence there is need for a reduction in the concentration of total cholesterol, triglyceride and LDL-cholesterol in the blood. Moreso, prior to the full manifestation of DM, individuals often exhibit an atherogenic pattern of risk factors that include lower level of HDL-cholesterol [45]. HDL-c function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease [46,47].

From this study, there was a marked increase in the lipid content which led to different lipid abnormalities in streptozotocin induced diabetic rat. Thus, it can be concluded from our findings that the administration of the ethanolic leaf extract of *C. albidum* due to its antihyperlipidemic activity, may be helpful in decreasing LDL-c, TC and TG which was actually elevated and also increase HDL-c, which was also decreased in hyperlipidaemic rats. In addition, its antihyperlipidemic effect could represent a protective mechanism against the development of atherosclerosis.

Thus, the significantly reduced serum insulin level recorded in the present study showed that STZ administration caused destruction to insulin producing β-cell in the pancreas. This finding has been reported by other investigators [48, 49]. Based on the appreciable increases in plasma insulin levels in rats treated with *C. albidum* extracts, it can be suggested that the possible mechanism of action of ethanolic extract

from *C. albidum* could be related to anti-oxidant activity that aids recovery from impaired glucose metabolism through release of insulin from the pancreas. *C. albidum* may serve as a significant indicator of its potential antioxidant activity in mopping up the free radicals produced by STZ.

CONCLUSION

In conclusion, extract of *C. albidum* has demonstrated strong hypoglycemic, antioxidant and anti-lipidemic and should be evaluated further to determine its possible mechanism(s) of action(s)

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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