

## Anti-Hyperglycemic and Hypolipidemic Effect of Ethanolic Extract of *Chrysophyllum albidum* Seed Cotyledon in Alloxan Induced-Diabetic Rats

<sup>1</sup>O.S. Olorunnisola, <sup>1</sup>S. Amao, <sup>1</sup>L.O. Ehigie and <sup>2</sup>A.F. Ajayi

<sup>1</sup>Department of Biochemistry, <sup>2</sup>Department of Physiology,  
Ladoke Akintola University of Technology, Ogbomosho, Nigeria

**Abstract:** The present study evaluate the anti-hyperglycemic and hypolipidemic effect of ethanolic extract prepared from *Chrysophyllum albidum* seed cotyledon in model of alloxan-induced diabetic rats. Results showed that the daily treatment of diabetic rats with ethanolic extract of *Chrysophyllum albidum* seed cotyledon twice daily for 7 days (100 and 200 mg kg<sup>-1</sup> orally) significantly decreased (p<0.001) the blood glucose levels by 11.92 and 12.10%, respectively in the treated induced diabetic rats compared to the diabetic control rats. The 100 mg kg<sup>-1</sup> dose of the extract showed insignificant decrease in the hepatic lipids (except HDL-cholesterol) concentrations, while the 200 mg kg<sup>-1</sup> of the extract showed a significant decrease (p<0.001) in the hepatic lipids (except HDL-cholesterol) in the treated diabetic rats and treated non-diabetic rats. However, the various doses of the extract significantly increased (p<0.001) the HDL-cholesterol in treated diabetic rats while, there was no significant effect on the hepatic HDL-cholesterol in the treated non-diabetic rats. The results justify the popular use of *Chrysophyllum albidum* seed cotyledon, pointing out to the potential benefit of the plant ethanolic extract in alternative medicine in the treatment of diabetes mellitus.

**Key words:** Diabetes Mellitus, *Chrysophyllum albidum* seed cotyledon, alloxan, hyperglycaemic and lipid profile

### INTRODUCTION

Diabetes Mellitus (DM) is a multifactorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities (Scoppola *et al.*, 2001) and altered intermediary metabolism of major food substrates (Unwin *et al.*, 2001). The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years (Bailey, 1999). Management of diabetes without any side effects is still a challenge for the medical system. This leads to an increasing search for improved anti-diabetic drugs.

Few of plants treatments used in traditional medicine for diabetes have received scientific scrutiny and the World Health Organisation has recommended that this area warrants attention (WHO, 1980). This research describes the study of *Chrysophyllum albidum* seed cotyledon (Sapotaceae). *Chrysophyllum albidum* (Linn.) belongs to the family Sapotaceae. It is primarily a forest tree species and its natural occurrences have been

reported in diverse ecozones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire (Bada, 1997). The plant often grows to a height of 36.5 m though it may be smaller (Bada, 1997). The african star apple fruit is a large berry containing 4-5 flattened seeds or some times fewer due to seed abortion (Keay, 1989). The plant has in recent times become a crop of commercial value in Nigeria. The fleshy pulp of the fruits is eaten especially as snack and relished by both young and old (Cenrad, 1999). The African star apple fruit has been found to have highest content of ascorbic acid with 1000-3,330 mg of ascorbic acid per 100 g of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew (Asenjo, 1946). As a medicinal plant, is commonly used as anti-microbial, antinociceptive, anti-inflammatory and antioxidant agent (Idowu *et al.*, 2003, 2006). However, no scientific investigation has so far been conducted on its anti-diabetic activity. The present work was therefore undertaken to study the anti-hyperglycemic and hypolipidemic effects of the ethanolic extract of *Chrysophyllum albidum* seed cotyledon in alloxan-induced diabetic rats.

## MATERIALS AND METHODS

**Plant materials:** *Chrysophyllum albidum* seed was, collected around the Southern Western part of Nigeria and then their voucher specimen deposited at Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria. The seed cotyledons were removed from the outer covering through crushing, air-dried and grounded into powder.

**Preparation of ethanolic extract of plant seed cotyledon:** Five hundred grams of powdered *Chrysophyllum albidum* seed cotyledon were first soaked for 30 min in 300 mL of hexane to de-fat. The extract was filtered and the residue was soaked in 900 mL of 70% ethanol for 5 days. The extract was filtered and concentrated at 78°C using rotary evaporator and further concentrated using water bath at 48°C.

**Animals:** Female albino rats weighing between 164-234 g were used. The animals were maintain under laboratory conditions humidity, temperature (23-25°C) and light 12 h light/dark cycle in the of Department Physiology animals house, Ladoke Akintola University of Technology, Ogbomoso, and allowed free accesses to grower mash and water *Ad libitum*. The animals were acclimatized for two weeks. The principle of Laboratory animal care guideline procedures were followed in the study (NIH publication revised, 1985).

**Induction of diabetes:** After fasting for 24 h the animals were given a single intra-peritoneal injection of freshly prepared alloxan solution using saline (0.9% (w/v) NaCl) as vehicle, at a dose of 12 mg alloxan/100 g body weight (Bahnak and Gold, 1982). The diabetic state was ascertained in terms of loss of body weight and high blood glucose levels. Symptoms of diabetes were observed within a week of alloxan injection.

**Experimental designs:** Thirty albino rats were used for the study. The rats were divided into 6 groups, each group consisting of 5 animals. The crude extract was dissolved in corn oil before administration to the rats. All administrations were done orally using oral cannula and were done twice daily (morning and evening) for a period of 7 days. Group I: Diabetic rats administered 100 mg kg<sup>-1</sup> of extract; Group II: Diabetic rats administered 200 mg kg<sup>-1</sup> of extract; Group III: Non-diabetic administered 100 mg kg<sup>-1</sup> of extract; Group IV: Non-diabetic rats administered 200 mg kg<sup>-1</sup> of extract; Group V: The diabetic control rats administered corn oil in place of the extract; Group VI: The normal control rats administered corn oil in place of the extract. After 7 days the experimental animals were subjected to overnight fasting and were sacrificed the following day.

**Preparation of serum:** A day (24 h) to sacrifice, food but not water was withdrawn. This was done to minimize the glycogen stored in the body. Animals were sacrificed by cervical dislocation and blood samples collected directly from the left ventricle of the heart to plain bottle. This was allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 min to obtain serum.

**Preparation of liver homogenate:** Livers from rats in each group were separately weighed and washed in 1.15% potassium chloride. The livers were homogenized in 0.25 M sucrose buffer at 4°C with a diluting factor of 4 using Teflon head homogenizer. The crude liver homogenate was centrifuged at a speed of 9000 rpm for 15 min at room temperature. The supernatants were stored at -20°C for biochemical assays.

**Biochemical assays:** Glucose levels was measured in serum using the glucose-oxidase method (Sera Pak, USA). Lipid profile (Triglycerides, total cholesterol concentrations as well as high-density lipoprotein cholesterol) were determined in liver homogenates using assay kits.

**Statistical analysis:** All values were expressed as Mean±SD. The differences were compared using one-way Analysis of Variance (ANOVA) followed by student t-test. p values <0.05 were considered as significant.

## RESULTS

In diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of ethanolic extract of *Chrysophyllum albidum* seed cotyledon, the serum glucose concentration was decreased by 11.92 and 12.10%, respectively. The percentage reduction in serum glucose concentration in diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract was significantly increased (p<0.001) compared to non-diabetic rats treated with the same concentration of the extract, diabetic control and normal rats. However, the effect of the extract was insignificant in non-diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract (Table 1).

Table 2, showed that the daily treatment of diabetic rats with ethanolic extract of *Chrysophyllum albidum* for 7 days (100 and 200 mg kg<sup>-1</sup>) insignificantly decreased the hepatic triglycerides, cholesterol and LDL-cholesterol concentrations compared to diabetic control rats.

The hepatic Triglycerides, Cholesterol and LDL-Cholesterol concentrations were significantly higher (p<0.05) in diabetic rats treated with 100 mg kg<sup>-1</sup> of the extract compared to diabetic rats treated with 200 mg kg<sup>-1</sup> of the extract. The hepatic triglycerides, cholesterol and LDL-cholesterol concentration were also high significantly (p<0.05, p<0.001) in diabetic rats treated with

Table 1: Effect of ethanolic extract of *Chrysophyllum albidum* seed cotyledon on serum glucose concentration in diabetic and normal rats

Groups	Treatment	Serum glucose concentration (mmol L <sup>-1</sup> )		
		Before administration	7 days after administration	Decrease(%)
1	Diabetic rats+100 mg kg <sup>-1</sup> extract	10.32±0.041	9.09±0.081	11.92*
2	Diabetic rats+200 mg kg <sup>-1</sup> extract	10.11±0.041	8.89±0.07	12.10*
3	Non-diabetic rats+100 mg kg <sup>-1</sup> extract	6.61±0.045	6.75±0.046	2.10
4	Non diabetic rats+200 mg kg <sup>-1</sup> extract	6.62±0.048	6.75±0.051	1.93
5	Diabetic control rats+0.1 mL corn oil	10.19±0.052	9.96±0.160	0.00
6	Normal control rats+0.1 mL corn oil	6.60±0.061	6.60±0.061	0.00

Values represent the means±S.D, (n = 5), \*Represent a significant decrease at (p<0.001) compared to the treated non-diabetic rats and diabetic control rats

Table 2: Effect of ethanolic extract of *Chrysophyllum albidum* seed cotyledon on Liver Triglycerides, Total Cholesterol, HDL-Cholesterol and LDL-Cholesterol concentrations in diabetic and normal rats

Group n = 5	Lipid Profile (mmol L <sup>-1</sup> )			
	Triglycerides concentration	Cholesterol concentration	HDL-Cholesterol concentration	LDL cholesterol concentration
1	2.38±0.41*	5.25±0.49*	0.25±0.07**	3.9182±0.7754*
2	1.94±0.27*	5.22±0.51*	0.41±0.09**	3.7282±0.9251*
3	1.32±0.19	3.34±0.40	1.25±0.15	1.3218±0.2615
4	1.69±0.28	3.00±0.37	1.21±0.13	1.1900±0.1385
5	2.53±0.46	5.42±0.58	0.20±0.02	4.1700±1.2519
6	1.69±0.28	3.87±0.46	1.37±0.23	1.4952±0.0984

Values represent the means±S.D. (n = 5), \* Represent significant decrease at (p < 0.05) compared to diabetic control rats, \*\* Represent significant increase at (p < 0.001) compared to diabetic control rats, 1– Diabetic rats treated with 100 mg kg<sup>-1</sup> of the extract, 2– Diabetic rats treated with 200 mg kg<sup>-1</sup> of the extract, 3– Non-diabetic rats treated with 100 mg kg<sup>-1</sup> of the extract, 4– Non-diabetic rats treated with 200 mg kg<sup>-1</sup> of the extract, 5– Diabetic rats without treatment (Diabetic control), 6– Non-diabetic rats without extract (Normal control)

100 and 200 mg kg<sup>-1</sup> of the extract compared to non-diabetic rats treated with the same concentration of extract. However, the hepatic triglycerides, cholesterol and LDL-cholesterol concentration was insignificantly reduced in non-diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract compared to the normal rats.

The hepatic HDL-Cholesterol concentrations was significantly higher (p<0.001) in diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract compared to the diabetic control rats. In the non-diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of extract, the hepatic HDL-cholesterol concentration was reduced, significantly compared to the normal control rats. Moreover, the HDL-cholesterol concentration of the normal control rats was significantly higher (p<0.001) compared to the treated diabetic rats and the diabetic control rats (Table 2).

## DISCUSSION

Diabetes mellitus is a metabolic disease associated with impaired glucose metabolism (Tallroth *et al.*, 1990), which adversely alters intermediary metabolism of lipids. (Mazzone *et al.*, 1984). As emerging evidence serves to confirm the pivotal role of blood cholesterol and particularly LDL-Cholesterol in the development of atherosclerosis related disease, it is timely to consider the importance of lipid and lipoprotein disorders in diabetes mellitus. In an individual patient, lipid and lipoprotein levels will depend on the extent of insulin deficiency or insulin resistance, hyperglycemia, obesity, diet and

presence of concomitant primary and secondary causes of hyperlipidemic state. In addition to absolute levels of lipid and lipoprotein, the composition of lipoprotein may be changed by diabetic state (Mazzone *et al.*, 1984).

In the present study, the anti-hyperglycemic activity of the ethanolic extract of *Chrysophyllum albidum* seed cotyledon was evaluated in alloxan induced diabetic and normal rats, using fasting plasma glucose test. The results show that serum glucose concentration in diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract was reduced significantly (p<0.001). The percentage reduction between 100 and 200 mg kg<sup>-1</sup> (11.92 and 12.10%, respectively) was insignificant making the anti-diabetic activities of the extract dose independent. However there was no significant reduction in the blood glucose of non-diabetic rats treated with the same concentrations of the extract (Table 1). The ethanolic extract of *Chrysophyllum albidum* seed cotyledon could therefore, exhibit antihyperglycemic effect rather than hypoglycemic effect. (Bailey *et al.*, 1985). The mechanism of action of ethanolic extract of *chrysophyllum albidum* seed cotyledon could be similar to biguanides such as metformin which is an antihyperglycemic compound; they do not affect the blood glucose concentration in normal state (Bailey *et al.*, 1985; Herman *et al.*, 1994; De fronzo and Goodman, 1995; Stumvoll *et al.*, 1995). The high ascorbic acid content may increase its antioxidant properties thereby reducing oxidative stress which has been implicated in etiopathogenesis of diabetes mellitus.

Furthermore, Lipid profile, which is altered in diabetic state (Betteridge, 1994), is one of the significant factors in development of cardiovascular diseases. Studies have shown that increased plasma triglyceride and cholesterol levels associated with diabetic state, may be a risk factor for vascular disease (Kamata and Yamashita, 1999; Kamata *et al.*, 2001; Shahar *et al.*, 2003). In this research, the hepatic triglycerides, cholesterol and LDL-cholesterol concentration of the diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of extract were lowered significantly ( $p < 0.05$ ) compared to the diabetic control rats. The diabetic rats treated with 200 mg kg<sup>-1</sup> of the extract have a significant reduction ( $p < 0.05$ ) of hepatic triglycerides, cholesterol and LDL-cholesterol concentration compared to diabetic rats treated with 100 mg kg<sup>-1</sup> of the extract. Moreover, the non-diabetic rats treated with the extract have a reduced hepatic triglycerides, cholesterol and LDL-cholesterol concentration compared to the normal control (Table 2).

The hepatic HDL-cholesterol concentration in diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract was significantly higher ( $p < 0.001$ ) compared to diabetic control rats. However, 200 mg kg<sup>-1</sup> of the extract give higher HDL-cholesterol compared with diabetic rats treated with 100 mg kg<sup>-1</sup> of the extract. More so, the hepatic HDL-cholesterol concentration of the normal control rats was slightly elevated than the non-diabetic rats treated with 100 and 200 mg kg<sup>-1</sup> of the extract. From this study, it could be deduced that the ethanolic extract of *chrysophyllum albidum* seed cotyledon exhibited a hypolipidemic effect in hepatic tissue. The hypolipidemic effect of the *Chrysophyllum albidum* seed cotyledon extract on lipid profile could be supported by its unsaturated fatty acid constituents, which amount to approximately 74% of the total fatty acids present in the cotyledon (Ajewole and Adeyeye, 1991).

Nonetheless, the anti-diabetic effect of the extract was not found to be dose dependent while it hypolipidemic effect was found to be dose dependent. The results however, indicated that the ethanolic extract of *Chrysophyllum albidum* possessed possible antihyperglycemic effect in alloxan induced diabetic rats with hypolipidemic potential.

However, further studies need to be carried out to investigate the active compounds, toxicity and possible mechanism responsible for the observed activities.

## REFERENCES

- Ajewole, K. and A. Adeyeye, 1991. J. Sci. Food Agric., 54 (2): 313-315.
- Asenjo, C.E., 1946. The high ascorbic acid content of the west Indian cherry. Science, 103: 219.
- Bada, S.O., 1997. Preliminary Information on the Ecology of *Chrysophyllum albidum* G. Don, in West and Central Africa. In: Proceedings of a National workshop on the potentials of the star Apple in Nigeria. Denton, O.A., D.O. Ladipo, M.A. (Eds.). Adetoro and M.B. Sarumi, pp: 16-25.
- Bahnak, B.R. and A.H. Gold, 1982. Effects of alloxan diabetes on the turnover of rat liver glycogen synthase. Comparison with liver phosphorylase. J. Biol. Chem., 257: 8775-8780.
- Bailey, C.J., 1999. New pharmacological approaches to glycaemic control. Diabetes Rev., 7: 94-113.
- Bailey, C.J., C. Day, S.L. Turner, B.A. Leaterdhale, 1985. Cerasee, a traditional treatment for diabetes, studies in normal and streptozotocin diabetic mice. Diabetes Res., 2: 81-84.
- Betteridge, D.J., 1994. Diabetic dyslipidemia. Am. J. Med. (Suppl. 6A), 96: 255-315.
- Cenrad, 1999. Centre for Environmental Renewable Natural Resources Management, Research and Development, Jericho, Ibadan. Publication No CEN. 011/1999 85.
- De Fronzo, R.A. and A.M. Goodman, 1995. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. New England J. Med., 333: 550-554.
- Idowu, T.O., G.O. Onawunmi, A.O. Ogundaini and S.A. Adesanya, 2003. Antimicrobial constituents of *Chrysophyllum albidum* seed cotyledons. Nig. J. Nat. Prod. Med., 7: 33-36.
- Idowu, T.O., E.O. Iwalewa, M.A. Aderogba, B.A. Akinpelu and A.O. Ogundaini, 2006. Biochemical and Behavioural effects of Eleagnine from *Chrysophyllum albidum*. J. Biol. Sci., 6 (6): 1029-1034.
- Kamata, K. and K. Yamashita, 1999. Insulin resistance and impaired endothelium-dependent renal vasodilation in fructose-fed hypertensive rats; Res. Commun. Mol. Pathol. Pharmacol., 103: 195-200.
- Kamata, K., N. Kanie and A. Inose, 2001. Mechanisms underlying attenuated contractile response of aortic rings to nor-adrenaline in fructose-fed mice. Eur. J. Pharmacol., 428: 241-249.
- Keay, R.W.J., 1989. Trees of Nigeria. A Revised Version of Nigerian Trees. Keay, R.W.J. and Onoche C.F.A. (Eds.). Stanfield DP. Clarendon Press, Oxford, 1 and 2: 476.
- Mazzone, T., D. Foster and A. Clait, 1984. *In vivo* stimulation of low density lipoprotein degradation by insulin. Diabetes, 33: 333-338.

- Scoppola, A., F.R. Montecchi, G. Mezinger and A. Lala, 2001. Urinary mevalonate excretion rate in type 2 diabetes: Role of metabolic control. *Atherosclerosis*, 156: 357-361.
- Shahar, E., L.E. Chambless, W.D. Rosamond, L.L. Boland, C.M. Ballantyne, P.G. Mc Govern and A.R. Sharnett, 2003. Atherosclerosis risk in community study, plasma lipid profile and incident ischaemic stroke, The Atherosclerosis Risk in Communities (ARIC) study, *Stroke*, 34: 623-631.
- Stumvoll, M., W. Nurjan, G. Periello, G. Dailey and J.E. Gerich, 1995. Metabolic effect of metformin in non-insulin-dependent diabetes mellitus. *New England J. Med.*, 333: 541-549.
- Tallroth, G., M. Lindgren, G. Sternberg, I. Rosen and C.D. Agadh, 1990. Neurophysiological changes during insulin induced hypoglycemia and in the recovery period following glucose infusion in type I diabetes mellitus and normal Men. *Diabetologia*, 33: 319-327.
- The WHO Expert Committee on Diabetes Mellitus, 1980. Technical Report Series 646. World Health Organization, Geneva, pp: 61.
- Unwin, N., E. Sobngwi and K.G.M.M. Alberti, 2001. Type 2 diabetes: The challenge of preventing a global epidemic. *Diabetes Int.*, 11: 4-8.