

LIPID PROFILE OF ALLOXAN-INDUCED DIABETIC WISTAR RATS TREATED WITH METHANOLIC EXTRACT OF ADANSONIA DIGITATA FRUIT PULP

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ABSTRACT

This study was carried out to assess the antidiabetic properties of Adansoniadigitata fruit pulp by evaluating the effect of its methanolic extract (MAD) on cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and triglycerides. Forty eight male wistar/albino rats weighing 160 to 240 g were randomly distributed into six treatment groups [Group 1, Normal control, each given only 0.2 ml distilled water daily for 4 weeks; Group 2, diabetes control rats, induced with 150 mg/kg b.w., i.p. administration of alloxan and thereafter given 0.2 ml distilled water throughout the study period; Groups 3, 4 and 5, diabetic (i.p., 150 mg/kg b.w. alloxan) rats were given single oral dose of MAD (100, 200 and 300 mg/kg b.w. respectively) for 4 weeks; Group 6, diabetic rats (i.p., 150 mg/kg b.w. alloxan) were treated with 84 mg/kg b.w. of Chlorpropamide, once daily for 4 weeks]. The serum concentration of cholesterol, HDL, LDL and triglycerides of all the animals in each group were determined after the 14th and 28th treatment. There was significant ($P < 0.001$) reduction of serum cholesterol, LDL and Triglycerides when compared with the diabetic control rats. These reductions were dose dependent and compared well with values obtained in the standard drug control group. Significant ($P < 0.001$) increase in HDL were seen in the MAD experimental groups but differed significantly ($P < 0.001$) with reduction seen in chlorpropamide group.

Keywords: Antidiabetic property, Adansoniadigitata fruit pulp, Lipid profile, Wistar Rats

INTRODUCTION

Diabetes mellitus, is a complex disorder characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action, is the most common serious metabolic disease in the world, affecting hundreds of millions and having incidence rate of about 1 % in industrialized countries (Stryer, 2000). There are three major types of diabetes (Stryer, 2000). Type I diabetes is usually diagnosed in childhood, hence called juvenile onset diabetes. In this diabetic type, the body makes little or no insulin and daily injection of insulin is needed. The exact cause is unknown and genetics, viruses, and autoimmune problems may play a role (Dyck, 2003). Symptoms of type I diabetes include; fatigue, increased thirst, increased urination, nausea, vomiting and weight loss in spite of increased appetite (Eisenberthet al., 2008). Type II diabetes, the commonest type of diabetes, occurs in adulthood, but young people are increasingly being diagnosed with this disease. Here, the pancreas does not make enough insulin to keep blood glucose levels normal, most often because the body does not respond well to insulin (Alemzadeh and Wyatt, 2010). This type of diabetes is being exacerbated by increasing occurrence of obesity, sedentary living and failure to exercise (Atkins and Brice, 1955) and often associated with symptoms such as blurred vision, fatigue, increased appetite, increased thirst and increased urination (Alemzadeh and Wyatt,

2010). Gestational diabetes which is the third type of diabetes is high blood glucose condition that develops at any time during pregnancy in non diabetic individuals. Women who have it are at high risk of type II diabetes and cardiovascular disease later in life (Dyck, 2003).

Treatment of diabetes centers around making available sufficient amount of insulin in the body system. Before the introduction of insulin therapy in 1922, the treatment of diabetes mellitus relied mainly on dietary measures which included the use of traditional plant-based therapies (Gray and Flatt, 1999). A number of plants with acclaimed antidiabetic properties are being studied in different laboratories throughout the world, especially in developing countries (Kameswaraet al., 1999). This became more apparent following WHO(1994) recommendations regarding the need to develop and evaluate better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta cells destruction, promoting beta cells regeneration or repair interrupting pathways leading to various complications of diabetes (WHO, 1994). These recommendations, together with the cost and side effects of most orthodox hypoglycaemic agents, have stimulated an increasing demand for natural products with anti diabetic activity that have fewer side effects (Kameswaraet al., 1999). The most promising of such products are of plant origin (WHO, 1994). The hypoglycemic properties of these plants used by traditional medical practitioners may be due to one or more of the arrays of chemical constituents of plant material. Some of these compounds may be toxic and thus the plant containing them, when consumed could confer varied levels of toxicity to the individual (Humphrey and Mckenna, 1997).

Baobab (*Adansonia digitata* L.) is a deciduous tree and belongs to the plant family called Bombacaceae. Baobab contains a number of substances usually employed for the treatment of numerous diseases in the African traditional medicine and for that reason it is also named "the small pharmacy" (Obizoba and Anyika, 1994). The dry baobab fruit pulp has a slightly tart, refreshing taste and is very nutritious, with particularly high values for carbohydrates, energy, calcium, potassium (very high), thiamine, nicotinic acid and vitamin C (very high) (Arnold et al., 1985). The fruit pulp has very high vitamin C content; almost ten times that of oranges. However, the vitamin C content of the bulk fruit pulp reportedly varies from 1623 mg/kg in one tree to 4991 mg/kg in another (Gebaueret al., 2002). Baobab fruit pulp has traditionally been used as an immunostimulant, anti-inflammatory, analgesic, antipyretic, febrifuge, and astringent in the treatment of diarrhoea and dysentery (Al-Qarawiet al., 2003).

The phytochemical analysis of methanolic extract of *Adansonia digitata* fruit pulp indicated the presence of glycosides, flavonoids, tannins, saponins, terpenoids and steroids (Mohammad and Hauwa'u, 2013). Recently, Mohammad and Hauwa'u, (2013) have shown that methanolic extract of *Adansonia digitata* fruit pulp

demonstrated antidiabetic effect by lowering blood glucose in alloxan induced diabetic rats. However, no work has been carried out on the effect of such treatment on lipid profile of alloxan induced diabetic rats to the best of our knowledge. It is in view of this that this study was designed to investigate the treatment effects of *Adansonia digitata* extract on the lipid profile of alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

Plant materials: The fruit pulps of *Adansonia digitata* were purchased from Kawo market, Kaduna State, Nigeria. The plant was identified and authenticated by a specialist in the Department of Biological Sciences, Kaduna State University

Extract preparation: The fruit pulps were broken, air dried and then crushed without seeds with a pestle and mortar and then sieved to obtain the powder. The powdered sample was soaked in methanol for 48hrs. The extract was obtained by filtration using a whatman filter paper No. 1. The methanol was evaporated using rotary evaporator. Solutions of the extract were prepared freshly for the study.

Chemicals used: All chemicals are of analytical grade obtained from Sigma (St. Louis USA) unless otherwise stated.

Experimental animals and alloxan induction: Forty eight albino rats weighing 160-240 g were purchased from the animal room of Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITR) Kaduna State. The rats were maintained under standard laboratory conditions and were allowed free access to both food and water throughout the period of the experiment. Stock solution of alloxan was prepared by dissolving alloxan monohydrate (0.9 g) in 6 ml of distilled water to give a stock concentration of approximately 150 mg/ml and diabetes was induced by single i.p. administration of alloxan monohydrate (150 mg/kg.b.w.). The rats with blood glucose level greater than 200 mg/dL, two days post-induction, were considered diabetic and used for this research work.

Experimental design: Forty eight rats were divided into six groups of eight rats each and were given the following treatments:

Group 1: normal control rats, given 0.2 ml of PBS

Group 2: diabetic control rats, given 0.2 ml of PBS after diabetic induction

Group 3: diabetic rats given fruit pulp extract (100mg/kg.b.w.) once daily

Group 4: diabetic rats given fruit pulp extract (200mg/kg.b.w.) once daily

Group 5: diabetic rats given fruit pulp extract (300mg/kg.b.w.) once daily

Group 6: diabetic rats given chlorpropamide (84mg/kg.b.w.) once daily

After the 14th and 28th dose of treatment with the extract, four rats were sacrificed from each group. Blood sample were collected in a centrifuge tubes for the estimation of total lipids (i.e. total cholesterol, triglyceride, LDL, HDL).

Biochemical analysis:

Serum cholesterol was estimated according to the method described by Trinder, (1969), serum triglycerides was estimated according to the method described by (Lothar, 1998). HDL-Cholesterol and LDL-Cholesterol was estimated according to the method described by Jacobs *et al.*, 1990.

Statistical analysis: The data was statistically analysed using GraphPad Instat3 Software (2000) version 3.05 by GraphPad Inc. Data are presented in Mean±Std. Statistical significance was accepted at a level of $p < 0.05$ and below.

RESULTS

Table 1 shows the serum lipid profile obtained after the 14th dose treatment with methanolic extract of the fruit pulp of *Adansonia digitata*. The results show that diabetes, induced by alloxan administration, significantly ($p < 0.001$) raised serum level of cholesterol, triglyceride and LDL. The serum HDL also appeared significantly higher ($p < 0.001$) than values of the normal rats except that its elevation in diabetic control rats, when compared with the normal control rat, is far lower than the individual values of other lipids. The results also revealed reduced serum level of cholesterol, triglyceride and LDL in the extract treated groups and these reductions were dose-dependent. The highest reduction seen in the highest dose administration (300 mg/kg b.w.) group even though these values were, however, significantly higher ($p < 0.001$) than those seen in the normal and Chlorpropamide control values. The serum levels of HDL, on the other hand, appeared significantly ($p < 0.001$) raised by the extract administration and again, this elevation was observed to be dose-dependent.

Table 1 : Serum lipid profile in alloxan induced diabetic rats after 14 oral doses of methanolic extract of fruit pulp of *Adansonia digitata* and Chlorpropamide.

Groups	Treatment Dose (mg/kgb.w.)	Serum Parameters			
		CHOL	TRIG	HDL	LDL
Normal control	PBS	68.16±2.10	50.82±2.12	40.30±1.11	20.86±0.65
Diabetic control	PBS	201.98±2.45	199.43±1.56*	54.92±1.03*	91.73±2.28*
MEFPAD	100	191.23± 1.21 ^e	193.07± 1.15 ^f	60.45± 0.98 ^m	82.82± 1.81 ^q
MEFPAD	200	182.08± 1.54 ^f	158.12± 1.20 ^l	75.78 ± 0.92 ⁿ	68.64± 2.36 ^r
MEFPAD	300	169.21± 1.63 ^g	120.53± 2.92 ^k	83.78 ± 1.02 ^o	52.04± 2.62 ^s
CP	84	150.65± 2.45 ^h	99.58 ± 1.12 ⁱ	91.17 ± 0.96 ^p	44.45 ± 0.73 ^t

Results are expressed as mean±SD, n=4. Values with asterisk in each column are significantly different at p<0.001 compared to normal control. Values bearing superscript in each column are significantly different at p<0.001 compared to diabetic control. MEFPAD: Methanolic extract of the fruit pulp of *Adansonia digitata*. CP: chlorpropamide

Table 2 shows the effect of the 28th dose treatment on the serum level of cholesterol, triglyceride, HDL and LDL. This was found to be significantly higher in diabetic control rats (p<0.001) when compared to normal control rats. The three groups orally administered with different doses of the extract had their serum levels of cholesterol, triglyceride, and LDL significantly (p<0.001) reduced in a dose related manner when compared with values in the diabetic control group but were, however, significantly (p<0.001) well above values of the normal control rats. The HDL serum level in extract-treated groups, on the other hand, were observed to be significantly higher (p<0.001) than what was observed in diabetic control rats. The serum level of cholesterol, triglyceride, and LDL in chlorpropamide treated rats was found to be significantly lower (p<0.001) with higher level of HDL (p<0.001) when compared to the three groups orally administered with different doses of the extract.

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The results (Tables 1 and 2) of the serum level of cholesterol, triglyceride, HDL and LDL in alloxan induced diabetic rats was compared after the 14th and 28th doses of oral administration with methanolic extract of *Adansonia digitata* fruit pulp. Significant decreases (p<0.001) were observed in the serum levels of cholesterol, triglyceride and LDL with higher level of HDL after 28th dose for each of the three groups administered with different dose and the group orally administered with chlorpropamide.

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Table 2: Serum lipid profile in alloxan induced diabetic rats after 28 oral doses of methanolic fruit pulp extract of *Adansoniadigitata* and Chlorpropamide.

Groups	Treatment Dose (mg/kgb.w.)	Serum Parameters			
		CHOL	TRIG	HDL	LDL
Normal control	PBS	67.12 ± 2.20	52.45 ± 1.91	37.58 ± 1.58	22.47 ± 2.34
Diabetic control	PBS	210.45 ± 1.81*	202.54 ± 2.48*	55.96 ± 1.70*	96.56 ± 1.99*
MEFPAD	100	179.76 ± 1.89 ^e	182.17 ± 2.12 ⁱ	69.14 ± 1.31 ^m	71.01 ± 1.43 ^q
MEFPAD	200	164.78 ± 1.94 ^f	151.27 ± 1.53 ^j	82.87 ± 1.54 ⁿ	56.13 ± 1.69 ^r
MEFPAD	300	157.45 ± 1.56 ^g	118.18 ± 1.89 ^k	95.46 ± 1.13 ^o	39.80 ± 1.18 ^s
CP	84	139.59 ± 1.45 ^h	87.34 ± 0.75 ^l	120.13 ± 1.23 ^p	28.50 ± 1.56 ^t

Results are expressed as mean ± SD, n=4. Values with asterisk in each column are significantly different at p<0.001 compared to normal control. Values bearing superscript in each column are significantly different at p<0.001 compared to diabetic control MFPEAD: Methanolic extract of the fruit pulp of *Adansoniadigitata*. CP: chlorpropamide

DISCUSSION

The present results showed high significant increases in plasma cholesterol, TG, and LDL in the diabetic rats, while HDL level was reduced. The treatment with the plant extract resulted in decrease in plasma cholesterol, TG and LDL level with increase in HDL level.

The significantly higher (p<0.001) serum lipids level observed in the alloxan induced diabetic control rats when compared to normal control might be as a result of disturbance in the regulation of the activity of the hormone-sensitive enzyme, lipase, by insulin due to its deficiency or absence, caused by the alloxan induced destruction of beta islet cells. Lipase is known to convert triglycerides to free fatty acids and glycerol. Insulin inhibits the hormone-sensitive lipase in adipose tissue and in the absence of insulin, the plasma level of free fatty acids increases. In liver, the free fatty acids are catabolized to acetyl CoA, and the excess acetyl CoA is converted to cholesterol, triglyceride and ketone bodies resulting in ketosis (Al-Shamaony *et al.*, 1994). The abnormally high concentration of serum lipoprotein in the diabetic control rats may also be due to increase in the mobilization of free fatty acids from the peripheral fat depots by glucagons in the absence of insulin (Pari and Latha, 2005). Excess of fatty acids in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids into triacylglycerol, phospholipids and cholesterol which may be discharged into the blood as lipoproteins (Bopanna *et al.*, 1997).

The significant (p<0.001) hypolipidemic activities shown by the methanolic extract administered orally in different doses when compared to diabetic control might be due to ability of the methanolic extract of *Adansonia digitata* fruit pulp to cause regeneration of the β-cells of the pancreas and potentiation of insulin secretion from surviving β-cells. The increase in insulin secretion and the consequent decrease in blood glucose level may lead to stimulation of fatty acid biosynthesis (Insulin stimulates lipid synthesizing enzymes (fatty acid synthase, acetyl-CoA carboxylase) and also the incorporation of fatty acids into triglycerides in the liver and

adipose tissue). In the presence of insulin, the hormone-sensitive lipase will be inhibited in the adipose tissue, and mobilization of fatty acid from adipose tissue by glucagons will also be inhibited and therefore leading to the observed decrease plasma level of free fatty acids (Best and Taylor, 1989).

The plasma concentration of Lipoprotein (VLDL and LDL) will reduce since there is no elevated level of fatty acids (from lipolysis and from breakdown of triacylglycerol by the hormone-sensitive lipase) that will be converted to acetyl CoA in the liver. Elevated acetyl CoA are converted to cholesterol, triacylglycerol, phospholipids and ketone bodies. The principal lipids carried by lipoprotein are triacylglycerol and cholesterol. Absence of elevated cholesterol and triacylglycerol in the liver will lead to decreased synthesis of lipoproteins. The cholesterol and triacylglyceride that will be transported will be from diet and *denovo* synthesis (Bopanna *et al.*, 1997). Therefore, it is notable that the reductions in plasma cholesterol levels observed in the treated groups were accompanied by significantly higher HDL level when compared to that of diabetic group. High levels of HDL have been reported to be inversely related to the incidence of coronary heart disease (Khan *et al.*, 2003). HDL may foster the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion. Also, high levels of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL. HDL plays a role in lipid metabolism, complement regulation and the immune response, it is also thought to carry excess cholesterol back to liver where it is converted to bile acids and excreted into the small intestine; because of this, HDL is often referred to as 'Good Cholesterol' with high levels associated with a decreased risk of myocardial infarction. HDL removes cholesterol from non-hepatic tissues to liver through the process known as reverse cholesterol transport (Khan *et al.*, 2003). Studies by Khan *et al.* (2003) have associated reduction in plasma HDL cholesterol in diabetic rats and diabetic patients to defect in reverse cholesterol transport.

A significantly lower ($p < 0.001$) serum level of cholesterol, LDL and Triglyceride was observed in the diabetic rats during the fourth week when compared to the first two weeks of oral treatment. However, it can be inferred that the methanolic extract of the fruit pulp of *Adansonia digitata* gave its most potent antidiabetic effect at the highest dose (300mg/kg) and after four weeks of treatment. Chlorpropamide, on the other hand, exert much higher antidiabetic activity than the most effective extract dose (300mg/kg).

The result of our study is in accord with the findings of other researchers (Claudia *et al.*, 2006 and Bopanna *et al.*, 1997) who reported that many plants extracts have potential therapeutic value in combating atherosclerosis which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level (Luka and Tijjani, 2013). The lipid abnormalities accompanied with premature atherosclerosis is the major cause of cardiovascular diseases in diabetic patients. Therefore, an ideal treatment for diabetes in addition to glycemic control should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes (Khan *et al.*, 2003). Several studies have shown that an increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also increases HDL-cholesterol (Luka and Tijjani, 2013). In the present study, the methanolic extract of the fruit pulp of *A. digitata* not only decreased the total cholesterol but also enhanced the HDL-cholesterol. High levels of TC and more importantly LDL cholesterol are major coronary risk factors (Das, 2003). Reduced TG and LDL associated with the administration of *A. digitata* extract to diabetic rats is the important finding of this experiment and support the traditional use of the plant in the management of diabetes.

CONCLUSION

This study has revealed the antidiabetic potential of the methanolic extract of the fruit pulp of *Adansonia digitata* and indicates that its methanolic fruit pulp extract exerts such antidiabetic effect by lowering serum lipids in alloxan induced diabetic rats and if used as a hypoglycemic agent, may also reverse dyslipidemia associated with diabetes and prevent the cardiovascular complications that are very prevalent in diabetic patients. Further studies on this plant will focus on bioassay-guided isolation of the active principles from this plant.

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