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***J. Nat. Prod. Plant Resour.*, 2012, 2 (2):239-243**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Evaluation of the antidiabetic property of aqueous extract of *Mangifera indica* leaf on normal and alloxan-induced diabetic rats

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ABSTRACT

The aqueous extract of mangifera indica leaf was administered orally at a dose of 400mg/kg body weight to both normal and alloxan-induced diabetic rats. Twenty four (24) rats divided into 4 groups of 6 rats in each group of which two groups were made diabetic and the other two groups were normal. One of diabetic groups was treated with the extract and the second served as diabetic control. The alloxan was given through intraperitoneal route at a concentration of 150mg/kg body weight. The administration of the extract lasted for 21 days. Effect of the extract on glucose, cholesterol, triglycerides, High density lipoprotein and protein concentrations were analysed. The toxic effect of the extract was determined using biochemical enzyme markers. The phytochemical screening of the aqueous extract and ethanol extract showed the presences of flavonoids, tannins, cardiac glycosides, resins, sterols, balsam and saponins. Treatment of the diabetic rats with the aqueous extract showed significant ($p<0.05$) reduction in the levels of glucose, cholesterol, triglyceride and enzymes activities therefore Mangifera indica aqueous extract showed that it possesses hypoglycaemic and hypolipidaemic properties and showed no toxic effect on the liver at the concentration employed and may be used for the management of diabetes mellitus.

Keywords: *Mangifera indica*, Alloxan, Diabetic Rats, Phytochemicals, Hypolipidaemic.

INTRODUCTION

Diabetes mellitus describe a metabolic disorder of chronic hyperglycaemia with disturbance of carbohydrate, fats, and protein metabolism resulting from defects in insulin secretion, insulin action or both [1]. It poses an increasing public health problem across the world. It is widely recognised that the prevalence of diabetes is rising rapidly [2]. There are more 12 million people with diabetes in the world as at 1997 and by 2010 this number is expected to approach 20 million [3]. The clinical significant of diabetes is often indicated by the presences of symptoms such as polyuria, polydipsia and unexplained weight loss and is confirmed by measurement of abnormal hyperglycaemia [4]. Long term complications of diabetes mellitus include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcer, amputation and charcoal joints. However, much of the clinical and economical toll of diabetes arise from complications of the diseases such as capillary basement, membrane thickening, nephropathy, neuropathy and accelerated arteriosclerosis [5][6].

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnosis, improve or treat physical and mental illness. Traditional medicines that have been adapted by other populations (outside its indigenous culture) are often termed alternative or complementary medicine. Herbal preparations and finished herbal products that contain parts of plants or other plants materials as active ingredients [7].The common is

cultivated in many tropical and subtropical regions and its fruit is distributed essentially worldwide. The leaves are ritually used as floral decorations at weddings, public celebrations and religious ceremonies. *Mangifera indica* grows up to 35-40m (115-130ft) tall, the trees is lived as some specimens still fruit after 300 years. It is used for many medicinal purposes as well as dietary functions. The leaves are used as a herbal remedy and when soak overnight and drink is reported to have antihyperglycemic activity [8].

MATERIALS AND METHODS

Collection of plants materials

The fresh leaves of the plant *Mangifera indica* (Mango) were obtained at University of Jos, Senior Staff Quarters environment and were authenticated at the Botany Department of University of Jos.

Experimental Animals

Twenty four young male Wister strain albino rats weighing between 150-185g were obtained from the Animal House Unit of University of Jos. They were maintained on animal feeds obtained from Grand Cereal and oil meals Jos, Plateau State.

Extraction of *Mangifera indica*

The fresh leaves of *Mangiferaindica* were cut with laboratory knife into smaller pieces, and then pounded with a mortar and pestle. The juice extract of *Mangiferai ndica* was obtained by further grinding using blender and then filter. The filtrate was kept in an oven at temperature of 60^{o}c until it was evaporated to dryness. The extract was pounded into powder form and kept in the desiccator until it was ready for used.

Phytochemical Screening

The extracts of *Mangifera indica* was screened for some phytochemical constituents using standard qualitative procedure [9].

Induction of Experimental Diabetes

Experimental diabetes was induced by a single intraperitoneal injection of alloxan at a dose of 150mg/kg. Diabetes was confirmed in the animal after 48 hours by estimation of blood glucose level. Animal with blood glucose level above 120mg/dl were selected.

Administration of the Extract

The *mangifera indica* leaf extract was given orally at a dose of 400mg/kg body weight daily. This was carried out for 21 consecutive days after the induction of diabetes in the rats.

Collection of blood sample

Blood samples were obtained from the optical plexus of the rats using non heparinized (plain) haematocrit capillary tubes. The samples were transferred to plain dried tubes, allowed to clot then centrifuge at 500rpm for 10 minutes to remove red blood cells and recover serum. The serum was used for the assays.

Experimental Grouping

The rats were divided into four groups of six animals each and are allowed to acclimatize for three days before the commencement of the study. The experimental groupings are A, B, C and D. Diabetes was induced in groups A and B by intraperitoneal injection of alloxan while the rats in groups C and D were not induced.

The grouping and administration of the extract is as follows

GROUP	TITLE	No of RATS	TREATMENT
A	Diabetes control	6	Feed + Water
B	Diabetes + Extract	6	Feed + Water + Extract
C	Normal control	6	Feed + Water
D	Normal + Extract	6	Feed + Water + Extract

Determination of Serum Glucose Levels

Glucose was determined after an enzymatic oxidation in the presence of glucose oxidase using kit product of Randox UK [10].

Determination of Serum Lipid profile

The determination of serum total cholesterol, triglyceride and high density lipoprotein levels was done using kit product of Randox, UK [11].

Determination of Serum Total Protein Levels
The total protein was determined using biuret method [12].

Determination of Enzymatic Activities
The determination of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities were done using kit product of Randox UK [13].

Statistical Analysis

All data are express as Mean $\pm$ Standard Deviation (SD). The result were analysed by one-way Anova and were applicable least significant differences (LSD) was used to determine significant result. Differences between groups were considered significant at $P<0.05$.

RESULTS

Phytochemical Screening
Table 1 shows the result obtained when the leaf extract was screened for phytochemicals. The aqueous extract shows the presence of flavonoids, tannins, saponins, cardiac glycosides, resins, sterols, balsam while ethanol extract shows the presence of flavonoids, tannins, cardiac glycosides, resins, sterols and balsam.

Table 1: Phytochemical screening of the extracts of *mangifera indica* leaf

Bioactive constituent	Chemical test	Aqueous extract	Ethanol extract
Alkaloids	Dragendrorff	-	-
Flavonoids	Lead acetate	+	+
Tannins	Ferric chloride	+	+
Saponins	General test	+	-
Cardiac glycosides	Salkowski	+	+
Resins	General test	+	+
Sterols	General test	+	+
Balsam	Alcoholic $FeCl_3$	+	+

Key: + => present, - => not present.

Serum Glucose Level
Table 2 shows the effect of *Mangifera indica* leaf extract on serum glucose levels. The aqueous extract of *Mangifera indica* has a significant ($P<0.05$) lowering effect on alloxan induced diabetic rats treated with 400mg/kg body weight of the extract.

Serum Total Protein Level
Table 2 shows the effect of aqueous of *Mangiferaindica* leaf on serum total protein level. The result shows that the diabetic control rats had significant ($P<0.05$) low serum total protein level compared with the normal control rats, while the diabetic treated showed a significant ($P<0.05$) increase in serum protein level compared to the diabetic control group.

Table 2: Effect of Aqueous Extract of *Mangiferaindica* leaf on Serum Glucose and Serum Total Protein

Animal grouping	GLUCOSE (mg/dl)	PROTEIN (g/l)
Diabetic Control	156.20± 2.83	8.53± 0.21
Diabetic + Extract	100.50±3.54[a]	13.11± 0.34[a]
Normal Control	104.14±1.21[a]	14.75± 0.39[a]
Normal + Extract	102.20±1.20[a]	14.91± 0.22[a]

Values are presented as ± SD, n=6
a = statistically significant when compared with diabetic control (P<0.05)

Serum Triglyceride Level
Table 3 shows the effect of the aqueous extract of *Mangifera indica* leaf on serum triglyceride levels of normal and alloxan-induced diabetes rats. The result showed significant ($P<0.05$) reduction of serum triglyceride levels in alloxan-induced diabetic rats administered with 400mg/kg body weight of the extract.

Serum Total Cholesterol
Table 3 shows the effect of the aqueous extract of *Mangiferaindica* leaf on serum cholesterol levels of normal and alloxan-induced diabetes rats. The result showed that aqueous extract of *Mangifera indica* had a significant ($p<0.05$)

reduction of serum total cholesterol levels in alloxan-induced diabetic rats treated with the extract compared to the diabetic group.

High Density Lipoprotein (HDL)

Table 3 shows the effect of the aqueous extract of *Mangifera indica* leaf on high density lipoprotein levels of normal and alloxan-induced diabetes rats. The result showed that aqueous extract of *Mangifera indica* had a significant ($p<0.05$) increase on high density lipoprotein levels in alloxan-induced diabetic rats treated with the extract compared to the diabetic group.

Table 3: Effect of Aqueous Extract of *Mangifera indica* leaf on Serum Lipid Profile Levels

Animal grouping	TG(Mmol/L)	T.C(Mmol/L)	HDL(Mmol/L)
Diabetic Control	4.95±0.35	1.85±0.02	0.55±0.07
Diabetic + Extract	3.35±0.07[a]	0.40±0.01[a]	1.96±0.10[a]
Normal Control	3.25±0.35[a]	0.41±0.03[a]	2.12±013[a]
Normal + Extract	3.20±0.01[a]	0.40±0.02[a]	2.13±0.20[a]

Values are presented as ± SD; n=6
a = statistically significant when compared with diabetic control (P<0.05)

Enzyme Activity

Table 4 shows the effect of aqueous extract of *mangifera indica* leaf on the activities of serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in normal and alloxan-induced diabetic rats. The result showed a significant ($P<0.05$) decrease in the activities of ALP, AST and AST in treated diabetics compared with the diabetic control group.

Table 4: Effect of Aqueous Extract of *Mangifera indica* leaf on the activities of serum enzymes (ALP, ALT, and AST) in normal and alloxan-induced diabetic rats

Animal grouping	ALP (U/L)	AST (U/L)	ALT (U/L)
Diabetic Control	123 .3± 2.83	18.2 ± 0.41	93.3 ±5.62
Diabetic + Extract	107.2 ± 1.41$_a$	13.5 ± 0.71$_a$	75.5 ± 3.54$_a$
Normal Control	111.5 ±2.19$_a$	14.1 ± 0.41$_a$	44.4± 4.24$_a$
Normal + Extract	110.4 ± 2.00$_a$	14.2± 0.12$_a$	44.1 ± 4.10$_a$

Values are presented as ± SD; n = 6
a = statistically significant when compared with diabetic control (P<0.05)

DISCUSSION

The Wister rats used were strictly males because it was reported that female sex hormones (17-βestradiol) has a lowering effect on the plasma cholesterol concentration [14]. Thus, using female rats may interfere with the accuracy of the serum cholesterol level, since it was one of the parameters analysed.

The phytochemical screenings of the plant extract (*Mangifera indica*) revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, resins, sterols, and balsam [15], reported that hypoglycaemic phytochemicals include alkaloids, glycosides, flavonoids and saponins. The work done by [16] also revealed that hypoglycaemic phytochemicals include flavonoids, tannins, alkaloids, steroids and trepenoids. The hypoglycaemic effects produced by *Mangifera indica* extract may be due to the presence of these bioactive constituents established in this study.

The extract at a dose of 400mg/kg body weight reduce significantly ($P<0.05$) the blood glucose level in alloxan-induced diabetic rats, which suggests that the plant may have hypoglycaemic effect. However, the mechanism of action of plant extract is yet unknown. Since alloxan is known to induce diabetes by destroying completely the pancreatic islet of β-cells which produces insulin, it is not certain that the *Mangifera indica* aqueous extract act by stimulating insulin release from the pancreatic β-cells [17][18]. It is likely that the extract produces its hypoglycaemic effect by acting as an analog of insulin and mimics some of the actions of insulin on glucose metabolism, such as enhancing up-take of glucose into the cells, inhibition of glucose absorption in the intestine as well as acting as anti-metabolites that are capable of blocking the pathway of fatty acid oxidation.

The extract significantly ($P<0.05$) decreased the serum cholesterol level in diabetic rats thereby suggesting *Mangifera indica* as a probably cholesterol lowering agent. The extract showed a significant reduction in the serum triglyceride level. A similar fall in serum cholesterol levels has been reported in patients during insulin therapy [19]. The hypercholesterolaemia observed in diabetic rats generally might be due to increased intestinal cholesterogenesis resulting from increased activity of β-hydroxy-β-methyglutaryl CoA (HMG-CoA) reductase in the intestine of Alloxan-induced diabetic rats as reported by Nakayama and Nagakawa [20], partly from the increased availability of

acetyl- CoA as a result of increased oxidation of fatty acids in diabetes mellitus. The fall in serum total cholesterol level of diabetic rats that received the plant extract further supports the hypoglycaemic effect of the *Mangifera indica* leaf.

Changes in serum enzymes levels are often early determinant of tissue damage either by toxicant or in disease conditions. Serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase are liver biochemical markers. However, the differences observed in the activities of these enzymes at the dose employed 400mg/kg body weight showed statistical significant reduction ($P<0.05$) as compared to diabetic control group. This implies that *Mangifera indica* at that concentration employed has no toxic effect on the liver of the rats. The increase in ALT activity in diabetes is always due to hepatocellular damage which is accompanied by AST activity [21]. The reversal AST and ALT activity in *Mangifera indica* treated diabetic rats towards normalcy is evidence of prevention of cellular and tissue damage in a diabetic condition [22].

CONCLUSION

In conclusion, the aqueous extract of *Mangifera indica* reduced the concentration of glucose, cholesterol, and triglyceride in experimentally induced diabetes rats. Therefore, the plant has a hypoglycaemic and hypolipidaemic effects on alloxan-induced diabetic rats. The study also showed that *Mangifera indica* extract may not have toxic effect on the liver at the employed dosage, seen in the lowered concentration of AST, ALT and ALP as biochemical enzymes makers of liver damage. Based on these findings, its use should be encouraged in the management of diabetes mellitus.

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