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Int. J. Biol. Chem. Sci. 10(4): 1573-1579, August 2016

International Journal of Biological and Chemical Sciences

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

In vivo hypolipidemic and hypoglycemic effects of aqueous extract of *Spondias mombin* leaves and detoxification of reactive oxygen species in alloxan-induced diabetic rats

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ABSTRACT

The effect of aqueous extract of *Spondias mombin* leaves extract on lipid peroxidation and antioxidant activity in alloxan induced diabetes was studied. Forty male albino Wistar rats (100-150 g body weight) were used. The rats were randomly selected into four groups containing 10 rats each. Group 1 was the control group and it was placed on normal rat chow. Group 2 was the *Spondias mombin* (spm) group placed on normal rat chow and given 250 mg/kg extract orally. Group 3 was the alloxan-induced diabetic (150 mg/kg) group (DM) and Group 4 was the diabetic group treated with 250 mg/kg extracts (Dm+spm). At the end of 30 days blood samples were collected by cardiac puncture and used for biochemical analysis. Results obtained revealed that blood glucose level in group 3 (Dm) was significantly higher ($p<0.05$) than control but the administration of *Spondias mombin* leaves extract significantly reduced the blood glucose level ($p<0.05$). Total cholesterol (TC), triglycerides (TG) and Low Density Lipoprotein (LDL) were significantly raised in the diabetic group while High Density Lipoprotein (HDL) was significantly reduced ($p<0.05$). Treatment with extract decreased TC, TG, LDL but significantly increased the HDL level ($p<0.05$). Lipid peroxidation was increased in the diabetic group and treatment with extract significantly reduced ($p<0.05$) the level of lipid peroxidation. Superoxide dismutase (SOD) and catalase (CAT) activities were decreased significantly in the diabetic group. Administration of extracts increased the antioxidant enzymes activities. The result suggests that aqueous extracts of *Spondias mombin* leaves possess hypoglycemic effects and improve lipid profile of diabetic rats. This effect may be secondary to its ability to reduce oxidative stress.

Keywords: Diabetes, SOD, CAT, Lipid peroxidation, hypolipidemic, hypoglycemic, *Spondias mombin*

INTRODUCTION

Most plants in every part of Africa are used for the treatment of different kinds of ailment and diseases. *Spondias mombin* is among the notable plants that play vital role in the management of various disease conditions. These conditions include cardiovascular diseases, dysentery, heamorrhoids, and diarrhea (Martinez, 2000). The plant leaves have been reported to contain antiviral

DOI : http://dx.doi.org/10.4314/ijbcs.v10i4.10

ellagtannins, caffenyl esters and phenolic acids (Corthout et al., 1994).

In traditional medicine, the extracts of fresh boiled leaf of *Spondias mombin* have been reported to be used in the treatment of dizziness, and are said to be strongly abortificient (Offiah and Anyanwu, 1989). Among the Phytochemical components of the leaves of *Spondias mombin* are alkaloids, flavanoids, tannins, saponins and phenolic compounds (Nnjoku and Akumefula, 2007). Flavanoids as antioxidant, they scavenge free radicals and prevents oxidative cell damage and also provides anticancer activity. Other basic function of the flavanoids includes its ability to protect against allergies, inflammation, platelet aggregation and ulcer (Okolie et al., 2008). According to Iweala et al. (2011), the seed of *Spondia mombin* possess hypoglycemic effects in alloxan induced diabetic rats. However nothing is known about the hypoglycemic effect of the leave extract of *Spondia mombin* in diabetic rats. Diabetes mellitus is a disease associated with defect in glucose metabolism (WHO, 1999) and is linked to oxidative stress induced by hyperglycemia (Iwaela et al., 2000) which manifest into hyperlipidemia and hypercholesterolaemia (Ross, 1999). It is estimated that about 3.2 million people died of diabetes related conditions annually. This situation is worst in developing countries where the number of people affected is expected to increase by 150% by 2030 (Hope, 2004). Management of diabetes mellitus usually involves adjustment of the patient lifestyle or in some cases supplementary with agents that lower blood sugar level. Recently attentions have been shifted toward the use of medicinal plant in the management of degenerative diseases such as diabetes mellitus. Therefore this study is designed to investigate the effects of aqueous leave extract of *Spondias mombin* on hypolipidemic, hypoglycemic and level of oxidative stress caused by alloxan-induced diabetes mellitus in Wistar rats.

MATERIALS AND METHODS

Preparation of leaf extract of *Spondias mombin*

The fresh leaves of *Spondias mombin* were collected, washed and dried in the laboratory for two weeks and the leaves ground to powder. The powdered *Spondias mombin* leaves were extracted by the bulk using the soxhlet extraction method. The liquid extract obtained was slowly evaporated in vacuo. The total yield was 28.06 g. The extract was used to prepare the required test concentrations. The extract was stored in the refrigerator at 4 ^{o}C.

Experimental design

Forty male albino Wistar rats weighing 100-150g were obtained from the Animal House of Department of Physiology, Cross River University of Technology, Okuku Campus, Nigeria. Four groups containing 10 rats each were randomly selected and used for the study. All the animals had access to feed and water *ad libitum*. Group 1 was the control (cont), fed on normal feed. Group two was the *Spondias mombin* (SPM) group fed on normal feed and administered with 250 mg/kg of extract. Group 3 was the alloxan induced (150 mg/kg) diabetic group untreated (DMU) and group 4 was the diabetic group treated with the extract (250 mg/kg). Diabetes was induced by intraperitoneal injection of 150 mg/kg bolus of alloxan. Accu check glucometer was used to confirm them diabetic after 3 days. At the end of 28 days, the animals were fasted for 12 hours, anesthetized with ether and blood samples collected via cardiac puncture and put into EDTA blood sample bottles for biochemical analysis.

Measurement of biochemical parameters

Blood samples were collected by cardiac puncture, put in EDTA sample bottle. Glucose oxidase method of Trinder (1969) was used for glucose determination.

To determine total cholesterol level in blood, Siedel et al. (1983) method was used. Enzymatic cholesterol colorimetric kit was used for analysis and absorbance measured at 550 nm against the reagent blank. Triglyceride was determined by the method of Sullevian et al. (1985). The equation of Friedwald et al. (1972) (LDL =TC-HDL+ TG/2.23) was used to estimate LDL-c level.

Protein was determined according to the method of Lowry et al. (1951) using standard protein solution. The method of Buege and Aust (1978) was used to determine lipid peroxidation. The levels of total superoxides dismutase was determined by the method of Misra and Fridovich (1972) while catalase activity was determine according to the method of Sinha (1971).

Data analysis

All data are presented as the means ± Standard Error of Mean (SEM). One way analysis of variance (ANOVA) was used for the analysis followed by a post-hoc Turkey's HSD test using the SPSS software package version 13.0. Means was considered to be significant at $p< 0.05$.

RESULTS

Blood glucose

As shown in Figure 1, blood glucose level was significantly ($p<0.05$) increased in alloxan-induced diabetic rats (115.86 ± 5.0 mg/dl) when compared with control (84.57 ± 3.0 mg/dl).

Following the oral administration of extract, blood glucose level decreased significantly ($p<0.05$) to 90.0 ± 2.0 in the DM+SPM group representing about 23% reduction.

Lipid profile

The TC in the diabetic untreated was significantly increased ($p<0.05$) when compared with the diabetic treated (Table 1). The mean triglyceride (mg/dl) in diabetic group was significantly ($p<0.01$) elevated compared with the control. Administration of extract to the diabetic group lowered the TC level significantly ($p<0.01$) by about 19.6%.

The mean level of low density lipoprotein (LDL-c) in the diabetic group was significantly ($p<0.05$) raised but lowered remarkably (70.8% and 26.36% respectively) following the administration of the extract. The high density lipoprotein cholesterol level (HDL-c) significantly ($p<0.05$) decreased in the diabetics when compared to control (Table 2).

Tissue lipid peroxidation and antioxidant enzyme

At the end of 28 days administration of extract, there was significant ($p<0.05$) increase in lipid peroxidation in the diabetic group compared to the control. Lipid peroxidation was significantly reduced in the diabetic treated and *Spondias mombin* group only (Figure 2).

Superoxide dismutase (SOD) activity was significantly ($p<0.05$) reduced in the diabetics compared to the control. There was a significant increase in SOD activity in both *Spondias mombin* group and the diabetics treated group (47.52%) when compared to the diabetic untreated group (Table 1).

Catalase activity was also significantly ($p<0.05$) lowered in the diabetic group compared to the control. In the *Spondias mombin* and diabetics treated groups, catalase activity was significantly ($p<0.05$) increased (25%) (Table 1).

Table 1: Effect of aqueous extract of *Spondias mombin* leaves on lipid profile and antioxidant activity in alloxan induced diabetes mellitus.

Parameters	CONTROL	SPM	DMU	DM+SPM
TC (mg/dl)	153.4 ± 3.0	143.66 + 3.0	176.46 ± 2.0*	154.56 ± 3.0
TG (mg/dl)	130.64 ± 4	118.09± 5.0	209.14 ± 15*	154.40 ± 13
HDL (mg/dl)	101.97 ± 4	107.73±10	87.79 ± 3*	118.34±1.3
LDL (mg/dl)	26.20± 8.0	21.57± 2.0	36.02± 2.0	10.49± 1.3
SOD (mol/g/protein)	13.16 ±1.2	9.71 ± 0.7	5.43 ± 0.8*	10.96 ± 1.1
Catalase (Kat f)	0.18±0.01	0.11 ± 0.02	0.06 ± 0.01*	0.10 ± 0.01

*p<0.05 significant different from control

SPM= *Spondias mombin* group, DM= Diabetic untreated group, DM+SPM (Diabetic treated with *Spondias mombin)*

Table 2: Effect of aqueous extract of *Spondias mombin* leaves on HDL/LDL ratio in alloxan induced diabetes mellitus.

PARAMETERS	CONT.	SM	DM	DM+SPM
HDL mg/dl.	101.97 ± 4.0	107.73 ± 1.4	87.79 ± 3.0	118.34 ± 1.3
LDL-c	26.20 ± 8.0	21.57 ± 2.0	36.02 ± 2.0	10.49 ± 1.0
Ratio	3.9 : 1	5.0 : 1	2.4 : 1	11.3 : 1

SPM= *Spondias mombin* group, DM= Diabetic untreated group, DM+SPM (Diabetic treated with *Spondias mombin)*

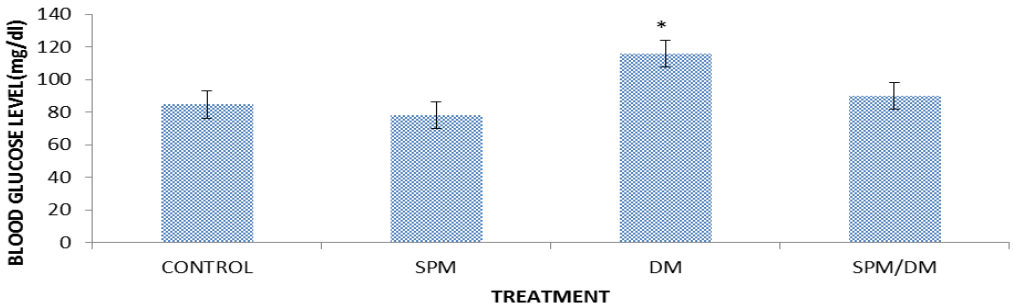

Figure 2: Fasting blood sugar following administration of *Spondia mombin* leave extract to diabetic rats. *p<0.05 significantly different from control.

SPM= *Spondias mombin* group, DM= Diabetic untreated group, SPM/DM= Diabetic treated with *Spondias mombin*

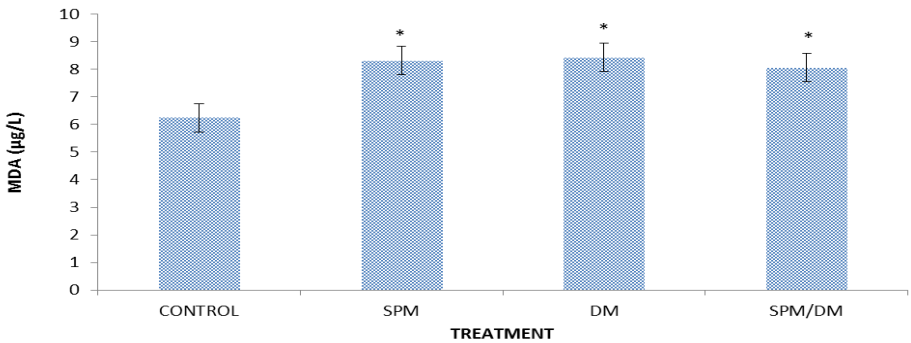

Figure 2: Serum MDA following administration of *Spondia mombin* leave extract to diabetic rats. *p<0.05 significantly different from control.

SPM= *Spondias mombin* group, DM= Diabetic untreated group, SPM/DM= Diabetic treated with *Spondias mombin*

DISCUSSION

The aim of the present study was to evaluate the effect of the oral administration of the aqueous extract of *Spondias mombin* leaves on lipid peroxidation caused by alloxan-induced diabetes and its antioxidant activity.

In this study, the results obtained showed that there was a significant decrease in blood glucose level and cholesterol level in the diabetic treated group suggesting an anti-hyperglycemic and hypolipidemic activity of the *Spondias mombin* leaves extract. Diabetes mellitus is a condition in which the regulatory mechanism of blood glucose in the body is distorted. Hyperglycemia either in humans or experimented animals cause some level of metabolic insults resulting from radical damage to tissues following production of reactive oxygen species and depletion of antioxidant enzymes (Soto et al*.,* 2002). The blood glucose level reduction shown in this study both in the groups administered with S*pondias mombin* extract and the diabetes treated groups suggest that there may be an active component in this extract that promotes blood glucose suppression probably via pancreatic B-cell regeneration, or insulin sensitivity of tissues. This observation is in agreement with the report of Iwaela et al. (2011), that the seed extract of *Spondias mombin* possess hypoglycemic effects in alloxan induced diabetic rats.

In this study, data presented shows that TG, LDL and VLDL-C levels were raised in the alloxan induced diabetic groups and also an increased TC level in the diabetic untreated group. Treatment with the leave extract significantly reduced the TC, TG, LDL and VLDL levels in the diabetics and normal rats. This result suggests that the leave extract may be having the component responsible for preventing the possible development of atherosclerotic plagues which forms a major risk factor in the development of high blood pressure (Wiebickia and Mikhailidis, 2002). The increase in HDL-c observed in this study is in variance with the earlier work by Ojiako and Nwanyo, 2006, who reported a decrease in HDL-C caused by the extract.

Lipid peroxidation in the diabetic treated and S*pondias mombin* groups were significantly reduced while catalase and superoxide dismutase enzyme activity in these groups were increased. In diabetes, reports have shown that auto-oxidation occurs leading to increased peroxidation and release of free radicals such as superoxide anions and reactive oxygen species (Soto et al*.,* 2002; Hink et al*.,* 2000). This free oxidants evokes chain reaction leading to depletion of antioxidant enzymes like SOD, catalase and glutathione peroxidase and cell damage (Cariello, 2006) Studies have shown that SOD, flavonoid and catalase are antioxidants that scavenges free radicals and prevent oxidative cell damage (Wu and Cederbaum*.,* 2003). From our result, the elevated SOD and catalase level and reduced peroxidation may therefore suggest that *Spondias mombin leave* extract elicit ROS scavenging and detoxification potentials as well as the capacity of preventing lipid peroxidation.

Conclusion

The result suggests that aqueous extracts of *Spondias mombin* leaves possess hypoglycemic effects and improve lipid profile of diabetic rats. This effect may be secondary to its ability to reduce oxidative stress.

COMPETING INTERESTS

Authors do not have any competing interests.

AUTHORS' CONTRIBUTIONS

EEN designed the study and he was involved in the evaluation of the biomarkers in conjunction with DEI. EEN also wrote the

first draft of the manuscript. SOJ handled data and statistical analysis in addition to correction of the first draft. GOU was involved in the evaluation of the biomarkers, handling of data and preparation of manuscript.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks Mr Ezekiel for his technical assistance.

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