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EFFECTS OF *ZANTHOXYLUM ZANTHOXYLOIDES* LEAVES ON BLOOD GLUCOSE, LIPID PROFILE AND SOME LIVER ENZYMES IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT
This study was carried out to investigate the biochemical effects of feed formulated with *Zanthoxylum zanthoxyloides* leaves in alloxan induced diabetic rats. Wister albino rats (n = 25) of either sex weighing 105-162g assigned into 5 groups (I-V) of 5 rats per group were investigated. Diabetes was induced in groups' I-IV by a single intraperitoneal injection of alloxan monohydrate (200 mg/kg body weight). Animals in groups' I-III received experimental feed containing 10%, 15%, and 20% dry powdered leaves of *Zanthoxylum zanthoxyloides* respectively for a period of three weeks, while those in groups IV and V received normal feed and acted as diabetic and non-diabetic controls respectively. Blood glucose and body weights of the rats were determined before and after treatment. Serum lipids {total cholesterol (TC), triglyceride (Tg), high density lipoprotein (HDL), low density lipoprotein (LDL)} and liver enzymes (AST, LDH, ALT & ALP) were determined by standard laboratory techniques. Significantly ($p < 0.05$) lower blood glucose was observed in the treated animals in comparison to non-treated groups. Total cholesterol and LDL were significantly lower in the treated groups, with lowest values found in group treated with higher concentration of the feed. It was also observed that LDH, ALP and ALT showed highest activities at 15% of the feed with AST activity lowest at the highest concentration of the feed. *Zanthoxylum zanthoxyloides* leaves though exhibits antidiabetic and hypolipidaemic effects, its uses at higher concentration needs further evaluation based on its actions on liver enzymes.

KEY WORDS: *Zanthoxylum zanthoxyloides*, antidiabetic effect, hypolipidaemia, liver enzymes, diabetic rats.

INTRODUCTION

Diabetes is a chronic metabolic disease that is showing an alarming increase in prevalence in developing countries such as Nigeria. Diabetes mellitus is a major metabolic syndrome characterized by derangement in carbohydrate metabolism associated with defect in insulin secretion or action. Medicinal plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world, especially in countries where access to the conventional treatment of diabetes mellitus is inadequate (Punitha, 2006). Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research (Daisy and Eliza, 2007; Noor *et al*. 2008). Various parts of medicinal plants including the roots, bark and leaves have been used for medicinal purposes, including the treatment of diabetes mellitus, stomachache, toothache, coughs, urinary and venereal diseases, leprous ulcerations, rheumatism, and lumbago, (Olatunji, 1983; Oliver- Bever, 1982).

Nigerian *Zanthoxylum* is a common plant found in the rain forest vegetation of southern Nigeria, and is represented by eleven species. A few of these species occur more abundantly in the savannah and dry forest vegetation of south- western Nigeria. The eleven *Zanthoxylum* species demonstrate very close similarities and relationships among themselves, and are identified as trees, erect shrubs or small tree, straggling or scandent shrubs or as a forest liana. Most traditional healers throughout Nigeria have used species of the *Zanthoxylum* for the treatment of a wide range of disorders, including toothache, urinary and venereal diseases, rheumatism and lumbago (Adesina, 1997). Metabolites isolated from *Zanthoxylum species* include alkaloids, aliphatic and aromatic amides, lignins, coumarins, sterols, carbohydrate residues, etc. Some of these metabolites have shown cytotoxic, molluscidal, anticonvulsant, anti-sicking, anesthetic, antibacterial, anti-hypertensive and anti-inflammatory properties. Crude aqueous extracts of the root bark of *Zanthoxylum zanthoxyloides* is used in folklore medicine for its anti-inflammatory activity. It is now well established by pharmacological study that the extract has anti-inflammatory activity (Oriowo, 1982). The present study was designed to evaluate the effect of feed prepared from *Zanthoxylum* leaves on blood glucose level, lipid profile and activities of some plasma liver enzymes.

MATERIALS AND METHODS

Animals: A total of 25 Adult Wister albino rats weighing 105g-162g obtained from the animal house of Pharmacy Department, University of Nigeria, Nsukka (UNN) were used. The rats were grouped into (5) – I, II, III, IV and V. Each group contains 5 rats and was fed twice daily, for 5 days with growers mash and water *ad libitum* for acclimatization. The initial glucose levels of the rats were determined using glucometer (ACCUTREND GC, Boerhinger Mannheim, Germany) before administration of alloxan using blood from tail tips. Before testing for blood glucose level or injection of alloxan to induce diabetes, the rats were fasted overnight (at least 12 h) but had free access to water.

Induction of diabetes: Diabetes was induced intraperitoneally in groups I-IV by injecting 200mg/kg body weight of alloxan dissolved in distilled water. The blood glucose levels of these rats were determined after 3 days of alloxan administration to confirm that diabetic condition has actually been induced, which was indicated by glucose level ≥ 7.8mmol/L.

Preparation of the experimental feed: *Zanthoxylum zanthoxyloide* leaves were collected in the farm land towards the end of harmattan season from Ugwulangwu in Ohaozara Local Government Area of Ebonyi State. These were identified and certified at Botany Department University of Nigeria, Nsukka.The leaves were sun dried to a constant weight and then ground with a mortar. The resulting powder obtained was sieved and stored in polyethylene bags at room temperature. Exactly 10g/90g, 15g/85g and 20g/80g of the ground leave/feed, representing 10%, 15% and 20% w/w was mixed together respectively. A little quantity of water was added to get them in a pellet form.

Feeding Experiment: While groups' I-III animals were fed with 10 %, 15 % and 20 % of the experimental feed respectively, animals in groups IV & V were fed with normal feed for a period of three weeks.

Biochemical analyses

At the end of feeding experiment (3 weeks), the animals were killed and blood samples collected for biochemical analyses. The blood samples collected into plain bottles were allowed to clot and retract after which samples were centrifuged at 2000g for 5 minutes and serum was separated thereafter. The method of Lopes-Veriella *et al*. (1977) was used to determine high density lipoprotein (HDL) while Triglycerides was determined using the method described by Tietz (1990).The method of Trinder (1969) was used to determine total cholesterol while Low density lipoprotein (LDL) was estimated using Frieldewald Equation (Frieldewald *et al*., 1972).Total protein (TP) was determined using colorimetric Biuret method as described by Weichselbaum (1995) and albumin was determined as described by Doumas *et al.* (1971). Reitman and Frankel Methodology (1957) were used to determine the transaminases. The standard methods according to the recommendations of the Deutsche Gesellschaft for Klinsche Chemie (1972) were used for the determination of lactate dehydrogenase (LDH) and alkaline phosphatase.

Data analysis

Data were evaluated using the SPSS/10.00 software. Results were expressed as Mean ± Standard Deviation. Comparison between groups was done using ANOVA. Values were considered statistically significant at 95% confidence level ($P<0.05$).

RESULTS

Table 1 show that there were reductions in body weight in the treated groups when compared with the non-diabetic and diabetic controls. However, more weight reduction was observed in diabetic group treated with 20 % of the experimental feed. For blood glucose (table 2), there were reductions in the treated groups in comparison with both the diabetic and non-diabetic controls, with highest effect observed in the group treated with 15 % of the experimental feed, where there was 21.7 % reduction in blood glucose.

The results showed that the levels of HDL and TG were highest at 20% of the feed but lower than values for non-diabetic and diabetic controls respectively. Also, significantly ($p<0.05$) lower levels of TC and LDL at higher concentration of the feed were observed. Albumin (Alb) and total protein (TP) were higher in the treated groups in comparison to the non-treated groups with the highest values observed at 15% of the feed (Table 3).

From table 4, LDH, ALP and ALT have the highest activity at 15% of the feed while AST activity was lowest at the same feed concentration. The activities of all the enzymes were higher in the treated groups when compared with the non-treated groups.

TABLE 1: Effect of *Zanthoxylum zanthoxyloides* Leaves on Body Weight of Alloxan Induced Diabetic Rats [1]

Body weight (g)	Group I	Group II	Group III	Diabetic control	Non-diabetic control
Initial	150 ± 1.0	131 ± 0.9	147 ±1.0	116 ±1.00	144 ±1.0
Final	147 ± 0.8 [a]	128 ± 0.9 [a]	140 ± 1.0 [a]	132 ±1.0 [b]	158 ±1.0
Change in body weight (%)	- 3 (2)	- 3 (2.3)	- 7 (4.8)	+16 (13.8)	+14 (9.7)

[1] Values are expressed as mean ± standard deviation (n = 5). Values with the same superscript horizontally are statistically ($p < 0.05$) different from diabetic control.

TABLE 2: Effect of *Zanthoxylum zanthoxyloides* Leaves on blood Glucose Level of Alloxan Induced Diabetic Rats [1]

Blood glucose concentrations (mmol/l)	Group I	Group II	Group III	Diabetic control	Non-diabetic control
Initial	8.7 ± 0.2	9.2 ± 0.2	9. 1 ± 0.2	8.9 ± 0.3	4.5 ± 0.2
Final	7.8 ± 0.1 [a]	7.2 ± 0.2 [a]	8.5 ± 0.1 [a]	8.8 ± 0.2[b]	4.7 ± 0.1
Change in blood glucose concentration (%)	-0.9 (10.3)	-2 (21.7)	- 0.6 (6.6)	+0.1(1.1)	+0.2(4.4)

[1] Values are mean ± standard deviation (n = 5). Values with the same superscript horizontally are statistically ($p < 0.05$) different from diabetic control

TABLE 3: Effect of *Zanthoxylum zanthoxyloides* on Serum Total Protein, Albumin and Lipid Profile in Alloxan Induced Diabetic Rats [1]

	Experimental Groups				
Parameters	Group I	Group II	Group III	Diabetic control	Non-diabetic control
HDL(mg/dl)	25.3±0.1[a]	21.9±0.1[a]	26.4±1.0[a]	23.1±4.5[b]	30.5±2.0
LDL(mg/dl)	34.9±0.1[a]	33.7±0.1[a]	20.7±0.1[a]	37.8±11.1[b]	32.9±5.6
TG(mg/dl)	33.6±1.0[a]	32.0±1.0[a]	34.7±0.2[a]	40.8±7.9[b]	42.5±10.3
TC(mg/dl)	67.1±0.1[a]	62.0±1.0[a]	54.1±0.1[a]	69.2±0.5[b]	71.9±1.0
TP(g/l)	5.8±0.1[a]	6.0±0.1[a]	5.6±0.1[a]	4.0±0.7[b]	5.3±0.6
Alb (g/l)	4.0±0.1[a]	4.2±0.1[a]	3.7±0.1[a]	3.1±0.7[b]	4.3±0.3

[1] Values are mean ± standard deviation (n=5)
Values with the same superscript horizontally are statistically ($p < 0.05$) different from diabetic control.
HDL: High density lipoprotein**, LDL:** Low density lipoprotein, **TG:** Triglycerides, **Alb:** Albumin **TC:** Total cholesterol, **TP:** Total protein.

TABLE 4: Effect of *Zanthoxylum zanthoxyloides* leaves on Serum Liver Enzymes of Alloxan Induced Diabetic Rats [1]

	Experimental Groups				
Liver Enzymes (IU/L)	Group I	Group II	Group III	Diabetic control	Non-diabetic control
LDH	202.0±13.0[a]	216.0±17.0[a]	211.0±19.0[a]	167.0±15.8[b]	140.0±21.7
ALP	132.0±7.0[a]	141.0±8.0[a]	137.0±7.0[a]	103.8±9.1[b]	101.0±6.2
ALT	11.3±1.0[a]	11.8±0.1[a]	10.9±0.0[a]	9.8±1.0[b]	7.1±1.0
AST	6.7±0.1[a]	6.2±0.1[a]	6.3±0.1[a]	5.4±0.4[b]	5.4±0.8

[1] Values are mean ± standard deviation (n = 5). Values with the same superscript horizontally are significantly ($p < 0.05$) different from diabetic control.
LDH: Lactate dehydrogenase, **ALP:** Alkaline phosphatase, **ALT:** Alanine aminotransferase, **AST**: Aspartate aminotransferase.

DISCUSSION

Traditional plants have been used for centuries in the treatment of diabetes (Okyar et al., 2001), but only a few have been scientifically evaluated. The present work has detected the antidiabetic and hypolipidaemic effects of *Zanthoxylum* leaves in alloxan-induced diabetic rats. Previous studies on *Zanthoxylum zanthoxyloides* have concentrated on the antibacterial effects (Mann Abdullahi *et al.*, 2010, Adegbolagun and Olukemi, 2010). Many plant leaves have been shown to exert hypoglycemic effect (Maghrani *et al.*, 2005, Jain *et al.*, 2010). Plants exhibit antidiabetic effects by a number of mechanisms, including enhancement of muscle glucose uptake and metabolism (Gray *et al*., 2000), enhancement of insulin production by regeneration of β-cells of the Islet of Langerhans (Ayber *et al.*, 2001 Jelodar *et al*., 2007, Yadav *et al*., 2008), possession of insulin-like substance (Gray *et al*. 2000), inhibition of insulinase activity (Gray *et al*., 2000), inhibition of renal glucose reabsorption (Maghrani *et al*., 2005), and inhibition of endogenous glucose production (Eddouks *et al.*, 2003).

Additionally, plant fibre has been found to interfere with glucose absorption (Nelson *et al.*, 1991). Some plants' bioactive products, such as γ-sitosteroline, fatty acids, anthraquinones, tannins and alkaloids have been claimed to have antidiabetic activity (Farswan *et al.*, 2009). For instance, it has been shown that β-sistosterol and tannin in leaf of such plants are responsible for their antidiabetic activity. Although phytochemical analyses were regrettably not done to identify the bioactive components of Zanthoxylum zanthoxyloides plant in the present study, we speculate that the hypoglycemia exhibited by diabetic rats treated with the experimental feed may be partly attributed to some of these bioactive products, particularly, tannins and sterol as tannins- and sterol-containing drugs have been shown to possess antidiabetic activity (Hatapakki *et al*., 2005). Flavonoids, chelerythrine, berberine and phenol canthine-6-one with strong antibacterial activity have been isolated from methanol

extract of the powdered root of *Z. zanthoxyloides* (Odebiyi and Sofowora, 1979; Tsuchiya et al. 1996), which by implication may be applied to the leaves, their roles in glucose metabolism are yet to be ascertained. Fibre-mediated mechanism may be an alternative way by which *Zanthoxylum zanthoxyloides* exerts its antidiabetic and hypolipidaemic effects on diabetic rats as plant leaves are richly endowed with a lot of fibres. Additionally, regeneration of β-cells cannot be ruled out as the probable mechanism by which *Z. zanthoxyloides* produced a significant reduction in blood glucose in the treated rats. This is because, improved blood glucose in the treated animals suggests either increased insulin release or improved insulin activity, both of which could be attributed to improvement in the integrity of β – endocrinocytes (Snigur *et al.*, 2008).

In addition to antidiabetic effect, *Z. zanthoxyloides* showed a beneficial effect in the maintenance of body weight as less weight gain was observed in animal fed with the experimental feed. This is also evidenced by improvements in serum lipids in the diabetic rats fed with diet supplemented with powdered leaves of *Z. zanthoxyloides* in comparison to the diabetic or non diabetic control rats. Interesting to note is the fact that diabetic rats fed with the experimental diet showed significant elevation in HDL-cholesterol, suggesting that the plant has protective effect on the heart. It is well known that in uncontrolled diabetes, the resultant increases in LDL, triglyceride and total cholesterol were associated with increased morbidity and mortality from coronary artery disease (Arvind *et al.*, 2002). The protective effect of *Z. zanthoxyloides* was seen to be extended to the liver in this study, as shown by increases ($p > 0.05$) in total protein and albumin, thus supporting the regenerating capacity of this plant leaves on the pancreatic tissues which are manifested by increased utilization of glucose and protein synthesis. However, it seems that this protective effect was at moderate (10%) concentration, as liver enzymes (LDH, and ALT) displayed increased activities at higher concentration (15%). We conclude that although *Z. zanthoxyloides* leaves exhibit antidiabetic and hypolipidaemic actions, its safety at higher concentrations needs to be confirmed in a well-designed study.

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