



The effect of ethanol extract of African *ficus glumosa* leaf on liver function in diabetic rats

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ABSTRACT

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Background: In Africa many plants and plant products are used traditionally for the managements of diabetes mellitus or its complication. But, science based evidence on some of this product safety need to be establish. **Objective:** We determine the effect of the Ethanol leaves extract of *Ficus glumosa* on fasting blood glucose level and liver enzymes of diabetes (DM) treated rats. **Methods:** Thirty (30) adult rats weighing (190 – 220) grams of about 8 to 10 weeks of age were used for this study in a standard laboratory setting. The animals were randomly divided into six groups (n= 5 rats /group) and diabetes was induced using single dose of alloxan 150 mg/kg intraperitoneal (ip). Group 6 served as the control group receiving distilled water (5ml/kg) alone via intra-peritoneal route (ip), Group 1 (DM+Distil water), 2, 3, 4 and 5 were given treatments for 7 days. Distilled water 5ml/kg, 100mg/kg, 200mg/kg and 400mg/kg of *Ficus glumosa* ethanol leaves extracts respectively via intraperitoneal ip while, Group 5 (DM+Insulin) received insulin 6.0 iu/kg. Before and after treatments Fasting blood sugar levels were measured. Rats were sacrifice for the Serum liver enzymes and liver tissue for histological study after the treatments. **Results:** The 100mg/kg and 400mg/kg of Ethanol leave extract of *Ficus glumosa* significantly lowered fasting blood glucose level and 400mg/kg show elevation in liver enzymes and reveal altered hepatocyte architecture (interphase hepatitis) compared with the control group 6 and lower dosage treatment group (2 and 3). **Conclusion:** The results of these study reveal that *Ficus glumosa* possesses hypoglycemic effect and at higher dose of 400mg/kg/day have effect on liver enzymes in diabetes rat.

KEY WORDS: Diabetes mellitus, liver enzymes, leaf extract, *ficus glumosa*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease with multiple etiology and many systemic complications, according to a recent estimate by the World Health Organization and International Diabetic Federation, There were 382 million people in the world with diabetes in the year 2013 and this is projected to increase to 592 million by 2035 [1]. This disease is associated with reduced life expectancy, significant mortality and diminished quality of life. In 2005 an estimated 1.1 million people died from diabetes and diabetes complications [1- 3]. Several studies have reported on the hypoglycemic property of *Ficus glumosa* in animal models of diabetes [4, 5]. They used organic solvent based extracts of the plant's stem bark but, the use of the leaf is the common practice in communities that have acclaimed to its hypoglycemic efficacy and it is therefore possible that the extracts may serve as a remedy by blocking or intercepting the activity of environmentally acquired toxins such as mycotoxins, insecticides and pesticides [6]. The changes in the intensity of the hypoglycemic effect of *Ficus glumosa* over a predetermined time course will be informative in establishing its anti-diabetic and medical value in prolonged usage. The hypoglycemic efficacy of *Ficus glumosa* has only been compared with those of oral hypoglycemic drugs in

the previous studies reviewed [7, 8]. Comparing its efficacy with that of insulin and the action on the liver enzymes will provide helpful information on its anti-diabetic benefits in type 1 DM although, Individuals with type-2 diabetes have a higher incidence of liver function test abnormalities. Liver function tests (LFTs) are commonly used in clinical practice to screen for normal liver physiology, monitor the progress of a known problem and effects of potentially hepatotoxic substances. Science based evidence to evaluate the chemical components, safety and effectiveness of traditional medicine need to be established for noble drug development. Although, research shows that some herbal medicine is effective for specific conditions further study of the safety and effective dose is still necessary [9-11]. The most common LFTs include the evaluations of serum Aminotransferases, Alkaline phosphatase (ALP), Aminotransferase (ALT) and Aspartate aminotransferase (AST). Mild chronic elevations of transaminases often reflect underlying insulin resistance [12] and loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production and hyperinsulinemia [13]. Hyperinsulinemia might directly lead to hepatic insulin resistance with associated increased lipogenesis and formation of fatty liver. The excess in free fatty acids found in the insulin-resistant state and the associated

oxidant stress from reactive lipid peroxidation and paroxysmal β -oxidation is known to be directly toxic to hepatocyte and leads to injury and elevation of serum transaminases [14]. However, elevation of transaminases does not always correlate with histological changes in the liver, the elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate impairment in insulin signaling rather than purely hepatocyte injury. In contrast, aspartate aminotransferase (AST) and γ -glutamyltransferase (GGT) serum concentrations are unrelated to changes in hepatic insulin action. Followed 451 non-diabetic Pima Indians for an average of 6.9 years to determine whether hepatic enzyme (ALT, AST, and ALP) elevations could be linked to the development of type-2 diabetes. After adjustment for age, sex, body weight, whole body insulin sensitivity and acute insulin response only elevated ALT. Research shows higher ALT as a risk factor for type-2 diabetes and indicates a potential role of increased hepatic gluconeogenesis in the pathogenesis of DM that presents with elevated ALT [13]. Many so called plant poisoning involves harmless species that have been treated with insecticides, weed killers, or fertilizers [15, 16]. Although no significant adverse effects have been reported for the local use of *Ficus glumosa* [17]. This study was to ascertain the hypoglycemic effect of ethanol leaf extract of Nigerian *Ficus glumosa* and its safety on the parameters of liver function test (ALT, ASP and ALP) in diabetic rats. We hypothesized that *Ficus glumosa* possesses hypoglycemic properties with no effect on liver enzymes if properly administered.

MATERIALS AND METHODS

Alloxan for the induction of diabetes (DM) was purchased from Sigma Aldrich (St. Louis, MO, USA) and Assay kits for the assessment of liver enzymes were purchased from (Randox Laboratory Ltd, North West Life Science Specialties Vancouver, Canada). Glucose-test Strips for assessment of plasma glucose levels were from (Accu-Check Advantage II, Roche. Diagnostics GmbH Germany). All other chemicals and reagents used were of analytical grade.

Collection of Plant Materials

Fresh leaves of *Ficus glumosa* plant was obtained locally from a farmer during dry season (Jan to April). The plant was identified and authenticated at the Herbarium Unit of Biological Science, Ahmadu Bello University Zaria, Nigeria and a voucher specimen (No. 0412) deposited for reference.

Animals

Thirty five (30) adult male Wister rats age 8 to 10 weeks (190-220) g were used in this study. The animals were kept in the cages under standard laboratory conditions with free access to food (standard pellets from Guinea Feeds, Plc., Nigeria) and water ad libitum. Rats were given one week to acclimatize under a standard laboratory environments.

Plant Extract preparation

The leaves of the plant were plucked from the stem and then dried in an oven during the month of October, 2014. The dried samples were pulverized using a mortar and pestle and extracted with 70% ethanol using cold maceration. The mixture was filtered and the filtrate concentrated under vacuum using rotary evaporator at 60 °C to obtain the Ethanol Extract Residue (EER). Ethanol Extract Residue (4g) was soaked in 99% ethanol (100ml) for twenty four (24) hours [19]. The extract was then filtered, using a filter paper (Whatman size no.4) into a beaker and later poured into a volumetric flask and Stoppard. The filtrate (4% w/v) was then stored at negative 4 °C in a refrigerator for later use. From the stock solution (4% w/v or 4000mg/100ml), volumes containing (400, 200 and 100) mg were determined per kilogram (kg) of animal weight.

Induction of Diabetes Mellitus

The rats were fasted overnight for at least 16 hours before induction of diabetes. DM was induced by a single intraperitoneal injection of freshly prepared alloxan 150 mg/kg body weight. While the control rats were similarly handled using 0.9% normal saline and served as the control group. The rats were then kept in a standard cages at room temperature with free access to food and water at libitum [20, 21, and 22]. The animal with fasting blood sugar level of ≥ 200 mg/dl are considered diabetes and used in this study.

Experimental Design

Group 1: DM receiving Normal saline (5ml/kg) diabetic control.

Group 2: DM+100mg extract receiving 100mg/kg body weight of *ficus glumosa*.

Group 3: DM+200mg extract receiving 200mg/kg body weight of *ficus glumosa*.

Group 4: DM+400 extract receiving 400mg/kg body weight of *ficus glumosa*.

Group 5: DM+6.0 IU Insulin receiving 6.0 iu/kg body weight short-acting insulin.

Group 6: Non water receiving Normal saline (5ml/kg) normal rats.

Animals (5 rats/ group) received the aforementioned treatments via intraperitoneal rout (ip) for a period of seven days.

Determination of blood glucose levels

Blood samples were collected from the tail vein of the rats on days before and after the treatments. Fasting blood sugar was done using glucose-oxidized principle sand results were expressed as mg/dl [12, 23].

Determination of liver enzymes

Serum Alkaline phosphatase (ALP) activity was assayed by the method of Bassey- lowry-Block (1946) with modification. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were assayed by the method of Reitman and Frankel (1957) with some modification.

Histological Studies

The liver samples fixed in Bouins fluid were processed and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken with digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK).

Statistical analysis

The data obtained were expressed as mean \pm standard error

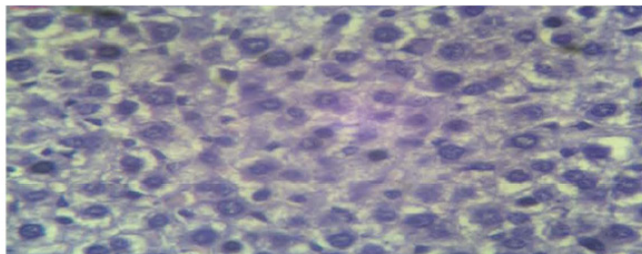
of mean (SEM) and data was statistically analyzed using SPSS Version 20.0. One way analysis of variance (ANOVA) with multiple comparisons (Dunnett's method) and value of P was < 0.05 .

RESULTS

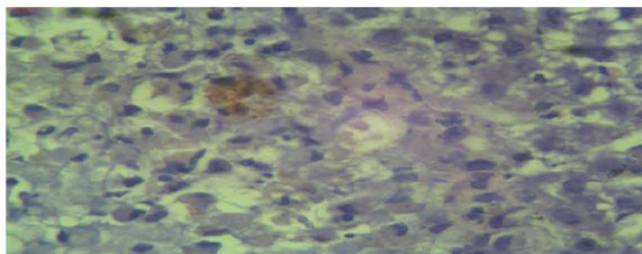
In this study, we found out the mean blood glucose of Non DM+Distil water control was invariable throughout the study period. On the contrary, the blood glucose of diabetic DM+Distil water untreated rats was significantly increased ($p < 0.05$) compared with the Non DM+Distil water normal and diabetic treated groups (Table. 1). The serum levels of liver enzymes were significantly up regulated in the DM+Distil water ($p < 0.05$) in the diabetic untreated group and histological study also revealed some findings (Tab. 2 & figure 1. Histology plates).



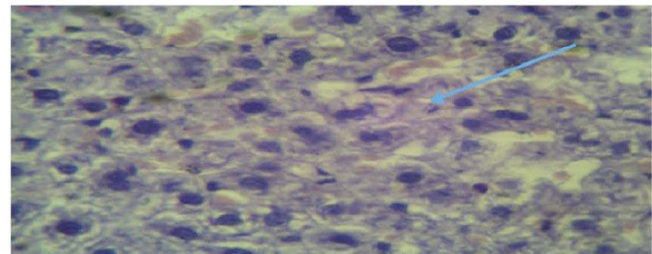
1. DM+Distil water. Section of the liver show preserved architecture with hepatocyte arranged in plates separated by sinusoid, the portal tract and central vein, appear normal. Conclusion: Normal liver histology.



2. DM+100 mg extract. Sections of the liver showed perceived architecture of the liver with hepatocyte arranged in plates separated by sinusoid. The portal tract and central vein appear normal. Conclusion: Normal liver



3. DM+200 mg extract. Section of the liver showed hepatic architecture with hepatocyte. Conclusion: Normal liver



4. DM+400 mg extract. Sections of the liver show mildly distorted architecture of the liver with hepatocyte arranged in plates separated by sinusoid, some of the hepatocyte showed micro vesicular steatosis with mild polymorph nuclear infiltrate in the portal triad and central vein appear normal. Conclusion: Inter-face hepatitis



5. DM+ 6.0 IU Insulin. Liver sections showed perceived architecture of the liver with hepatocyte arranged in plates separated by sinusoid. The portal triad and central vein appear normal. Conclusion: Normal liver.



6. Non DM+Distil water. Sections of the liver showed perceived architecture of the liver with hepatocyte arranged in plates separated by sinusoid. The portal triad and central vein appear normal. Conclusion: Normal liver.

The histopathological study results of the liver samples obtained from each of the six group, some changes are observed in the group that received 400mg of extract as seen (plate 4).

Figure 1. Plates. Showing the histological results for all the experimental groups at $\times 100$.

Table 1. Fasting blood glucose level (FBG) before and after treatments in (Mean \pm SEM)

Animal Groups	Initial (Before) mg/dl	Final (After) mg/dl
1.DM+Distil water	481.5 \pm 32.3*	465.0 \pm 44.3*
2.DM+100 mg Extract	423.3 \pm 33.1*	202.0 \pm 20.5*
3.DM+200 mg Extract	475.2 \pm 34.3*	331.3 \pm 38.0*
4.DM+400 mg Extract	468.0 \pm 19.4*	205.5 \pm 23.1*
5.DM+6.0 IU Insulin	484.0 \pm 32.6*	307.0 \pm 33.2*
6.Non DM+Distil water	098.4 \pm 03.8	085.6 \pm 03.7

Fasting blood glucose were significantly different * $P < 0.05$ vs. Non DM+Distil water; $\dagger P < 0.05$ vs. DM+Distil water.

The analysis of variance (ANOVA) showed that on day 0 before the starting treatments (distil water, extract and insulin), fasting blood glucose (FBG) levels were not significantly different in all the diabetes groups. However, on day seven after starting treatment FBG were significantly decreased in all the groups that received the different doses of extracts compared with the untreated group (DM+Distil water) which showed sustained elevation in FBG. Blood glucose levels were significantly lower in the extracts treated and DM+ 6.0 IU Insulin compared with the DM+Distil water (Table 1).

The analysis of variance (ANOVA) showed no significant difference in the serum levels of AST, ALP and ALT of the Non DM+Distil water and the DM+Distil water groups. The dose of 400mg/kg of the extract significantly had elevated AST, ALP and ALT compared with all the groups including DM+Distil water and DM+6.0 IU Insulin groups (Table 2). Moreover, the histopathological study results of the liver samples obtained from each of the six group, some histological changes suggestive of interphase hepatitis are observed in the group that received 400mg of extract (figure 1. Histology plate 4).

DISCUSSION

This study was aimed to evaluate the effect of ethanol leaf extract of Nigerian ficus glumosa on fasting blood glucose and liver function test results in alloxan induced diabetic rats. Diabetes Mellitus (DM) is characterized by multiple metabolic complications such as changes in normal food metabolism, altered enzymes functions and diabetes

ketoacidosis (DKA) that continue to be the major cause of morbidity and mortality rate in developing countries [2, 13, 24]. Hyperglycemia remains the hallmark and the diagnostic indicator of DM [25]. The baseline fasting blood sugar levels before the commencement of the treatment were significantly increased compared with the normal rats (Non DM+Distil water) in all the diabetic groups. Subsequently, following the administration of the treatments the FBG on the 7th day were significantly reduced in the extracts treated groups and insulin treated group compared with the DM+Distil water group (Table. 1). These results suggest that, Ethanolic leaves extract of *Ficus glumosa* possesses hypoglycemic, this is in line with previous study that reported on the hypoglycemic effect of African *Ficus glumosa* [4]. The hypoglycemic effect of this extract may be due to the presence of some active components like Flavonoids, ceramides, saponins, Cardiac glycosides and steroids [18, 26]. These result showed that, the hypoglycemic effects at (100 and 400) mg/kg is time dependent compared with 200mg/kg and insulin.

However, we observed that there were no significant differences between Non DM+Distil water and DM+Distil water groups in the liver enzymes assessed except for AST and elevation in AST is not specific indicator of liver parenchyma damage, as it is present in other tissue (red blood cells, cardiac, skeletal muscle). However, serum AST, ALT and ALP were all elevated in the group that received 400mg/kg of the extract compared with the other groups (plates). The liver enzymes elevation need to be further evaluated to rule out alloxan effect on the liver hepatocyte [31] This result suggested leakage of these enzymes into the serum may be due to hepatocyte damage caused by 400mg/kg of the extract, more investigation need to be carried out to ascertain the exact action of ficus glumosa on hepatocytes. Putting together the results of liver enzyme (ALT, ASP, and ALP) assessments and the histology result of the liver samples obtained in the present study (Table.2 & figure 1. Histology plates), the liver sample from the group that received 400mg/kg showed mild fatty change and interphase hepatitis (figure 1. Plate 4). These effects may be as a result of the higher dosage of the extract 400mg/kg affecting the hepatocytes evidence from the total liver enzymes elevation [16, 32].

Table 2. The liver enzymes results of alloxan induced experimental rats treated groups

Treatment	Liver enzymes (IU/L) (mean \pm SEM)		
	AST	ALT	ALP
DM+Distil water	21.21 \pm 1.62*	32.60 \pm 2.21ns	69.60 \pm 3.01ns
DM+100mg/kg Extract	ns 18.40 \pm 4.40	ns 32.60 \pm 8.20	\dagger 63.00 \pm 16.0
DM+200mg/kg Extract	ns 18.80 \pm 2.86	ns 36.80 \pm 3.71	ns 70.20 \pm 3.51
DM+400 mg/kg Extract	\dagger 24.60 \pm 1.78	\dagger 39.60 \pm 3.14	\dagger 77.60 \pm 3.70
DM+6.0IU Insulin	ns 22.80 \pm 3.31	\dagger 37.20 \pm 3.89	ns 71.40 \pm 3.40
Non DM+Distil water (control)	18.00 \pm 1.30	33.60 \pm 2.44	66.80 \pm 4.81

* $P < 0.05$ vs. Non DM group; $\dagger P < 0.05$ vs. DM+Distil water group; ns (not significant)

CONCLUSION

The results from this study indicate that *Ficus glumosa* possess anti-hyperglycemic effect and the intensity of the effect appears to increased with dose. However, at higher dose the extract are seen to be hepatotoxic evidence by the significance increase of the liver enzymes (AST, ALT and ALP), mild fatty changes and interphase hepatitis seen with the higher dosage of the extract.

Further biochemical and pharmacological investigations would be required to know the chemical compositions and mechanism of action of the Nigerian *Ficus glumosa* which we are currently involved.

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AUTHOR CONTRIBUTIONS

Mahaneem Binti Mohamed (general monitoring); Umar Zayyanu Usman experimentation, manuscript writing. All the authors participated in the data analysis.

CONFLICTS OF INTEREST

Conflicts of interest declared none.

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