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Antidiabetic and Toxicity Evaluation of Aqueous Stem Extract of *Ficus asperifolia* in Normal and Alloxan-Induced Diabetic Albino Rats

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ABSTRACT

toxicity properties of Ficus asperifolia aqueous stem extract on normal and alloxan-induced diabetic rats was evaluated in this study There was a significant decrease ($p< 0.05$) in blood glucose concentration, serum triglyceride, Total cholesterol, Low Density Lipoprotein and Very Low Density Lipoprotein and a significant increase ($p< 0.05$) in concentration of High Density Lipoprotein of the extract-treated animals(i.e. animals that received 400mg/kg, 800mg/kg and 1200mg/kg body weight doses) when compared to the diabetic control animals. There was a significant increase ($p< 0.05$) in serum AST activity in all the animals that received the various doses (i.e. 400mg/kg, 800mg/kg and 1200mg/kg body weight) of the extract and also in the ALT activity for the animals that received the various doses for 7 days and those that received the 1200mg/kg body weight dose of the extract for 21 days. The concentration of serum albumin and total protein significantly fluctuated in the animals while a significant increase ($p< 0.05$) was observed in serum total and conjugated bilirubin concentration in most of the experimental animals. Results obtained in this study show that aqueous stem extract of Ficus asperifolia at the doses used possesses antidiabetic properties but may be toxic to the living system.

Keywords: *Diabetes; Toxicity; Alloxan; Ficus asperifolia, Bilirubin*

INTRODUCTION

Diabetes can be defined as a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins. The complications of diabetes include vascular diseases, eye disorders, renal disorders and a host of secondary infections [1]. Diabetes mellitus remains a global major health problem in the World over with the tropics inclusive. In the past decade, the United States has recorded a 33% rise in the cases of diabetes [2]. Incidence and prevalence of type 2 diabetes are increasing globally; the World Health Organization estimated that in year 2000, 171 million people had diabetes, representing 2.8% of the world's population, and predicts that this number will increase to 366 million (4.4%) by 2030 [3].

The treatment of diabetes with synthetic drugs is generally not preferred because of its high cost and the range of side effects caused. Hence development of traditional or alternative medicine is needed. This is why the use of herbs has more than tripled over the last 10 years [4]. Herbal drugs constitute an important part of traditional medicine and literature shows that there are more than 400 plant species showing antidiabetic activity [5].

Ficus asperifolia (sand paper tree) which belongs to the *Moraceae* family is a small or average size tree, terrestrial or epiphyte which can reach 20m in height. [6]. *F. asperifolia* is abundant in the savannah regions, especially along river banks and marshy areas at an altitude of up to 1100m. The leaves are enormous and displayed spirally, the limb is largely oval or has a form of ellipse and the roots are most often fibrous [6]. It is one of the many highly medicinal plants with a variety of uses which includes its use as analgesic, anti-tumors, anti-cancer, diuretic, abortificients, ecobolics and menstrual cycle pain reliever [6]. It is also used for treating nasopharyngeal infections, oedema, gout and veneral diseases [6]. It is found in Senegal, Uganda, Tanzania, Natal (South Africa), Madagascar and Cameroon [6]. It also found across many states in Nigeria.

Watcho *et al.* reported that *F. asperifolia* induced uterotonic effect in the estrogenized isolated rat uterus which justifies the traditional use of the plant for its abortificient and contraceptive properties [7]. Research conducted by Nkafamiya *et al*. revealed that the leaves of *F. asperifolia* consumed in Michika, Hong and Song Local Government Areas of Adamawa State, Nigeria contain substantial level of nutrients and could contribute useful amount to human diet. The mineral composition of this non-conventional leaf vegetable also showed that it could be a rich source of minerals. The anti-nutritional content of the leaves are below the established toxic level and implies that, the overall nutritional value of the vegetable will not be affected [8].

F. asperifolia is a medicinal plant that has been employed for treatment of a variety of ailments. The aim of this research is to find out if this plant can be used for the treatment/management of diabetes and also to check for possible toxic effect on the living system.

MATERIALS AND METHODS

Eighty- five male albino rats of norvegicus strain weighing (200 - 250g) were obtained from the Animal Holding of Benue State University, Markurdi, North Central Nigeria. *F. asperifolia* stem was obtained from Icheke Village of Omala Local Government Area of Kogi State, North Central Nigeria and was appropriately identified at the Forestry Department of Ministry of Agriculture and Natural Resources, Lokoja, Kogi State, Nigeria. Assay Kits for Aspartate aminotransferase and Alanine aminotransferase were products of Randox Laboratories, United Kingdom. All other reagents used were of analytical grade and were all prepared in all glass distilled water.

Experimental Design

85 rats were housed in standard cage and allowed to acclimatize to Animal House for 7 days. They were fed with normal rat pellet and tap water throughout the experiment. Animals were handled according to the international ethical guide-lines for the care of laboratory animals throughout the experiment. The rats were divided into 2 groups. The first group containing 25 rats was used for the antidiabetic study and was further divided into 5 (i.e. A, B, C, D, and E). Group A served as the normal control which was administered with distilled water. Group B served as the diabetic control that was also administered with distilled water while Groups C, D and E were diabetic rats treated with 400mg/kg, 800mg/kg and 1200mg/kg body weight doses of the extract respectively. The second group contained 60 rats which were used for the toxicity evaluation. Rats were further divided into 4 groups (F, G, H and I). Group F served as the control and was administered with distilled water while groups G, H and I received 400mg/kg, 800mg/kg and 1200mg/kg body weight doses of the extract respectively. The rats were sacrificed 24 hours after administration for 1, 7 and 21 days.

Preparation of Aqueous extract of *Ficus asperifolia* stem

The stem of *F. asperifolia* was first weighed after which it was cut into pieces and oven dried at 40^0C to a constant weight. The dried stem pieces were ground to powder using an electric grinding machine. The powder (100g) was percolated in 1 litre of distilled water with constant shaking and kept in the refrigerator for 48 hours. It was thereafter filtered using Whatman No 1 filter paper and the filtrate concentrated on a water bath at 80^0C.

Induction of Diabetes

A single 150mg/kg body weight dose of alloxan monohydrate in saline solution was administered intraperitoneally having fast the animals for 12hours. Normal rats received the same volume of 0.9%w/v saline solution through the same route. The animals were returned to their cages after injection and allowed free access to food and water. After 4 days, the fasting blood glucose concentrations were measured from tail blood samples by using a One Touch Ultra® glucometer (Lifescan; Johnson & Johnson, Milpitas, CA, USA). Animals with blood glucose concentrations above 180mg/dl were used for the experiment [9].

Blood Collection

Blood samples for glucose level determination were collected from the tail tip of the rats. To obtain serum for Liver function indices analysis, the rats were anaesthetized in a jar containing cotton wool soaked in chloroform, they were then sacrificed by jugular puncture and their blood collected in an unheparinized bottle and allowed to stand for 10 minutes to clot. Serum was then collected using a Pasteur pipette.

Enzyme, Protein and Liver Function indices Measurements

Serum triglycerides, total cholesterol and HDL were estimated by enzymatic colorimetric end point methods using Span diagnostic reagent kit. LDL and VLDL were obtained by calculations using the formula provided in cholesterol diagnostic kit booklet. Aspartate aminotransferase (AST) (EC 2.6.1.1) and Alanine aminotransferase (ALT) (EC 2.6.1.2) activities were assayed at 546nm [10]. Serum Total Protein concentration was determined at 540nm using the Biuret method [11]. Serum total and conjugated bilirubin were analysed at 540nm [12]. Serum albumin determination

was done using the method of [13] at 639nm. The blood glucose levels were determined for all the samples by the glucose-oxidase method [14]. All measurements were done using spectronic 21 digital Spectrophotometer (Bausch and Lomb, Rochester NY). The data were subjected to statistical analysis. All significant differences were determined by ANOVA and were complemented by Duncan's multiple range tests.

RESULTS

Table 1 shows the effect of aqueous stem extract of *F. asperifolia* on blood glucose concentration of diabetic albino rats. All the diabetic animals treated with the various doses of the extract showed a significant decrease in blood glucose concentration ($p<0.05$) when compared to the diabetic control rats. The obtained results were not significantly different from the normal control animals.

The effect of the extract on the lipid profile parameters of diabetic rats is presented in Table 2. All the diabetic treated animals showed a significant decrease ($p<0.05$) in the concentrations of serum triglyceride, total cholesterol, LDL and VLDL and a significant increase ($p<0.05$) in serum HDL concentrations when compared to the diabetic control animals.

The effect of administration of aqueous extract of *F. asperifolia* stem on Rat Serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities is presented in Table 3. There was a significant increase ($p<0.05$) in the activity of AST for all the experimental animals when compared with the control. AST activity increased with increase in dose for animals that received the extract for 1 day while it decreased with increase in dose after 7 and 21 days administration. ALT activities in Rats that received the various doses of the extract for 7 days and those that received the 1200mg/Kg dose of the extract for 21 days also increased significantly ($p<0.05$) when compared to the control group.

The effect of the extract on the serum albumin and total protein concentrations is shown in Table 4. Experimental animals that received the 400mg/Kg dose of the extract for 1 day and those that received the 800mg/Kg and 1200mg/Kg doses for 21 days showed a significant decrease ($p<0.05$) in serum albumin concentration when compared with the control animals while animals that were administered with the 1200mg/Kg dose for 1 day showed a significant increase ($p< 0.05$) in serum albumin concentration. Similar result was obtained for serum total protein. Experimental animals that were administered with the 400mg/kg dose for 1 day showed a significant decrease ($p< 0.05$) in serum Total protein concentration. However, after administration of this dose for 21 days, the concentration of serum Total protein increased significantly ($p<0.05$) in the rats when compared to the control rats. The effect of aqueous extract of *F. asperifolia* stem on Serum Total and conjugated Bilirubin is presented in Table 5. There was a significant increase ($p< 0.05$) in serum Total bilirubin in the animals that received the 800mg/Kg and 1200mg/Kg doses of the extract for 1 day and those that received 400mg/Kg and 800mg/Kg doses of the extract for 7 days. All the animals that received the various doses of the extract showed significant increase ($p<0.05$) in serum Conjugated bilirubin concentration except the animals that received the 800mg/Kg dose for 21 days. The observed increase in serum Conjugated bilirubin was dose-dependent.

Table 6 shows the result of qualitative phytochemical screening of aqueous stem extract of *F. asperifolia*. Alkaloids, Flavonoids, Resins, Cardiac glycosides, Terpenes and Steroids were detected while Tannin, Saponin, Balsam and Phenols were absent.

Table 1: Effect of *Ficus asperifolia* aqueous stem extract on blood glucose concentration of alloxan-induced diabetic albino rats.

DAYS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
1	86.67±6.50^a	183.00±46.60^b	99.00±42.33^a	91.30±22.81^a	86.00±8.18^a
2	86.67±6.50^a	183.00±46.60^b	89.00±21.63^a	91.66±4.16^a	85.00±9.16^a
3	86.67±7.23^a	184.33±38.73^b	88.00±21.51^a	75.67±6.42^a	73.00±12.00^a
4	87.00±4.00^a	188.33±43.09^b	92.3±23.18^a	82.00±8.88^a	82.33±9.81^a
5	88.00±7.54^a	179.00±47.47^b	87.67±23.35^a	91.67±4.16^a	85.00±9.16^a
6	89.67±3.05^a	169.33±26.08^b	97.67±16.77^a	92.00±3.00^a	89.67±3.05^a
7	88.33±4.04^a	171.67±26.63^b	92.00±14.73^a	93.33±4.72^a	85.30±9.60^a

Concentrations are expressed in mg/dL ±SD; n=5, values with different superscripts are significantly different at $p<0.05$. A and B represent normal control and diabetic control rats respectively and were administered with 1cm^3 of distilled water. C, D and E represent diabetic animals that were treated with 400, 800 and 1200mg/kg body weight dose of the extract respectively.

Table 2: Effect of *Ficus asperifolia* aqueous stem extract on Lipid profile of alloxan-induced diabetic albino

	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
TRIGLYCERIDE	13.67±1.08^{a}	149.20±5.91^{b}	55.00±1.89^{c}	81.38±2.49^{d}	95.33±2.17^{e}
TOTAL CHOLESTEROL	52.67±1.78^{a}	141.99±7.67^{b}	97.33±3.27^{c}	75.67±2.28^{d}	92.00±2.37^{e}
HDL	25.00±2.16^{a}	10.26±2.78^{b}	46.33±1.79^{c}	35.33±2.58^{d}	23.33±1.49^{a}
VLDL	2.73±0.22^{a}	29.84±0.55^{b}	11.00±0.38^{c}	16.27±0.50^{d}	19.07±0.42^{e}
LDL	24.93±0.50^{a}	100.90±3.96^{b}	40.00±2.60^{c}	24.07±3.08^{a}	49.60±1.86^{d}

Concentrations are expressed in mg/dL ±SD; n=5, values with different superscripts are significantly different at p<0.05. A and B represent normal control and diabetic control rats respectively and were administered with 1cm^{3} of distilled water. C, D and E represent diabetic animals that were treated with 400, 800 and 1200mg/kg body weight dose of the extract respectively.

Table 3: Effec of administration of aqueous stem extract of *Ficus asperifolia* on male rat Serum Aspartate aminotransferase and Alanine aminotrasferase activities.

DAYS OF EXRACT ADMINISTRATION	SERUM AST				SERUM ALT			
	F	G	H	I	F	G	H	I
1	58.33±1.53^{a}	87.67±48.79^{ab}	125.00±5.19^{bc}	137.67±2.31^{c}	171.67±0.58^{a}	201.00±85.74^{a}	191.33±8.14^{a}	175.67±59.4^{a}
7	58.33±1.53^{a}	145.00±1.73^{b}	132.33±2.08^{c}	127.00±1.73^{d}	171.67±0.58^{a}	199.33±1.53^{b}	196.00±2.64^{b}	186.67±3.21^{c}
21	58.33±1.53^{a}	165.33±3.05^{b}	139.00\±2.64^{c}	113.33±3.05^{d}	171.67±0.58^{a}	171.76±0.58^{a}	171.33±1.53^{a}	177.00±4.36^{b}

Enzyme activities are expressed in UI ±SD; n=5, values with different superscripts are significantly different at p<0.05. F represents control rats that were administered with 1cm^{3} of distilled water, G, H and I represent animals that were administered with 400, 800 and 1200mg/kg body weight dose of the extract respectively.

Table 4: Effect of administration of aqueous stem extract of *Ficus asperifolia* on male rat Serum Albumin and Total Protein concentrations.

DAYS OF EXRACT ADMINISTRATION	SERUM ALBUMIN				SERUM TOTAL PROTEIN			
	F	G	H	I	F	G	H	I
1	3.53±0.12^{a}	2.33±0.25^{b}	3.53±0.25^{a}	3.93±0.02^{c}	7.00±0.10^{ac}	6.20±0.44^{b}	6.60±0.44^{bc}	7.53±0.25^{a}
7	3.53±0.12^{a}	3.40±0.05^{a}	2.40±1.73^{a}	3.50±0.32^{a}	7.00±0.10^{a}	7.50±0.01^{a}	7.20±0.99^{a}	5.800±2.070^{a}
21	3.53±0.12^{a}	3.61±0.08^{a}	3.24±0.06^{b}	3.29±0.01^{b}	7.00±0.10^{a}	7.50±0.00^{b}	7.13±0.23^{a}	7.10±0.00^{a}

Concentrations are expressed in g/L ±SD; n=5, values with different superscripts are significantly different at p<0.05. F represents control rats that were administered with 1cm^{3} of distilled water, G, H and I represent animals that were administered with 400, 800 and 1200mg/kg body weight dose of the extract respectively.

Table 5: Effect of administration of aqueous stem extract of *Ficus asperifolia* on male rat Serum Total Bilirubin and Conjugated Bilirubin concentrations.

DAYS OF EXRACT ADMINIS TRATION	SERUM TOTAL BILIRUBIN				SERUM CONJUGATED BILIRUBIN			
	F	G	H	I	F	G	H	I
1	0.10±0.01^{a}	0.16±0.06^{a}	0.29±0.02^{b}	0.35±0.02^{b}	0.05±0.01^{a}	0.16±0.05^{b}	0.18±0.02^{b}	0.28±0.03^{c}
7	0.10±0.01^{a}	0.24±0.12^{b}	0.23±0.02^{b}	0.18±0.02^{ab}	0.05±0.01^{a}	0.07±0.02^{b}	0.08±0.00^{b}	0.08±0.00^{b}
21	0.10±0.01^{a}	0.23±0.03^{a}	0.16±0.06^{a}	0.42±0.38^{a}	0.05±0.01^{a}	0.77±0.01^{b}	0.06±0.01^{a}	0.07±0.00^{c}

Concentrations are expressed in µmol/L ±SD; n=5, values with different superscripts are significantly different at p<0.05. F represents control rats that were administered with 1cm^{3} of distilled water, G, H and I represent animals that were administered with 400, 800 and 1200mg/kg body weight dose of the extract respectively.

Table 6: Qualitative Phytochemical screening of aqueous stem extract of *Ficus asperifolia*

Phytochemicals	Status
Alkaloids	+
Flavonoids	+
Tannins	-
Saponins	-
Balsams	-
Cardiac Glycosides	+
Terpenes and steroids	+
Resins	+
Phenols	-

KEY
Present = +, Not Present = -

DISCUSSION

Diabetes is a disease condition characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack [15]. Alloxan also acts by destroying the beta cells [16]. Intraperitoneal administration of alloxan to rats in this study led to a significant increase in blood glucose concentration, a result that is consistent with several studies in rats [17-19]. Administration of the aqueous extract however caused a significant decrease in blood glucose concentration of the alloxan-induced diabetic rats to normal levels. This may be attributed to hypoglycemic and/or insulin-mimetic properties of the aqueous stem extract of *F. asperifolia*. It may also be due to the ability of the extract to cause regeneration of dead beta cells or induction of insulin secretion by surviving beta cells as concluded in the experiment carried out and published by Mohammed et al. (2010)[17].

In diabetes mellitus, insulin deficiency causes excessive mobilization of free fatty acids and under utilization of chylomicrons and VLDL, leading to hypertriacylglycerolemia [20]. Elevated levels of cholesterol present in VLDL or LDL are associated with atherosclerosis, whereas high levels of HDL have a protective effect [20]. In this study, diabetic animals that received the various doses of the extract had a significantly reduced triglyceride, cholesterol, LDL and VLDL concentrations and a significantly increased HDL concentration than the diabetic untreated animals. The results obtained were however significantly different from the normal control animals and show that there was uptake of plasma lipids for storage which was made possible by either the insulin-mimetic property of the plant or by insulin produced by extract-regenerated beta pancreatic cells.

Medicinal plants elicit their actions due to the phytochemicals present in them. Qualitative phytochemical analysis of

F. asperifolia stem detected the presence of alkaloids, flavonoids, cardiac glycosides, resins, terpenes and steroids. The presence of these phytochemicals may be responsible for the hypoglycemic and hypolipidemic properties of *F. asperifolia* observed in this study. Garzon de la Mora *et al*. reported that alkaloid extract of *Lupin exaltatus* decreased glycemia and blood cholesterol in alloxanised Wister rats [21]. In the same vein, Mahanimbine (carbazole alkaloid from *Murraya koengii* leaves reduced the elevated fasting blood sugar, triglycerides, low density lipoprotein and very low density lipoprotein levels in diabetic rats while it increased high density lipoprotein level at doses of 50 and 100mg/kg(i.p.)[22]. It has also been reported that flavonoids extracted from lotus (*Nelunbo nucifera Gaertn*) leaf at doses 50 and 200mg/kg for 28days significantly decreased fasting blood sugar, serum total cholesterol and triglyceride levels and increased high density lipoprotein level in alloxan-induced diabetic mice [23]. Moreover, E and Z guggulsterones (active ingredients in gugulipid extracted from the resin of *Commiphora mukul*) showed differential effect with a significantly improved Perixisome Proliferator-activated Receptor (PPAR) gamma expression and activity in in vivo and in vitro conditions respectively. It also exhibited 3T3-L1 preadipocyte differentiation in vitro, results suggesting guggulsterone has both hypoglycemic and hypolipidemic effect which can help to cure type II diabetes [24]. Though the scope of this research did not involve the isolation of phytochemicals present in *F. asperifolia* and the elucidation of their mechanism of action, their presence however confirms the hypoglycemic and hypolipidemic activity observed in the plant and the obtained results are similar to those observed in earlier studies by [21-24].

The liver is prone to xenobiotic-induced injury because of its central role in the metabolism of foreign compounds and its portal location within the circulatory system [25]. All types of hepatitis (viral, alcoholic, drug-induced, etc.) cause hepatocyte damage that can lead to elevations in the serum ALT activity. This study showed that aqueous extract of *F. asperifolia* stem did not have any significant effect on ALT activity on first administration. The significant increase in ALT activity observed after administration of extract for 7 days can be attributed leakage from most especially the Liver or any of the other principal sources not checked in this study [26]. However, after 21 days of extract administration, animals that received the 400mg/Kg and 800mg/Kg doses of the extract were able to recover from the effect the extract. This may be attributed to adaptation of the animals to the extract at those doses. The liver also contains considerable amount of AST, where it is associated with Liver parenchyma cells. AST is important in the diagnosis of heart and liver damage caused by heart attack, drug toxicity, or infection although it is not as specific a liver marker enzyme as ALT. The increase observed in serum AST activity can be attributed to leakage from the Liver and other principal sources into the extracellular fluids occasioned by plasma membrane derangement by the constituents of the extract leading to excessive leakage of cytosolic materials [26].

Albumin is a major protein of human plasma and represents about 25% of total hepatic protein synthesis and half its secreted proteins. Its synthesis is depressed in a variety of diseases, particularly those of the liver [20]. In this present study there was a fluctuation in serum albumin concentration which shows that the liver's synthetic ability and ability to maintain nutrient homeostasis was compromised as a result of administration of the extract. The result obtained for total protein is similar to that of albumin and further confirms compromise of liver functions. Animals after 7days administration of the extract attempted to recover from the effect of the extract. This could not be achieved due to the extract's overwhelming effect occasioned by its continued administration.

Bilirubin is a product of hemoglobin catabolism where 1g of hemoglobin is estimated to yield 35mg of bilirubin [20]. The increase in total bilirubin observed in this study may be attributed to either overproduction of bilirubin as a result of excessive breakdown of hemoglobin or an impairment of the bilirubin excretion mechanism. The increase in serum conjugated bilirubin shows that the conjugation process is intact but however points to the inability to effectively excrete conjugated bilirubin. This may be attributed to reflux of bilirubin into the blood stream as a result of bilary obstruction [20].

CONCLUSION

The results obtained in this research work shows that aqueous stem extract of *F. asperifolia* at the doses tested possesses antidiabetic properties but may be toxic to the living system and should therefore be used with caution.

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