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Assessment of the anti-hyperglyceamic effect of *Ficus asperifolia* plant leaf aquous extract on alloxan-induced diabetic rats

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ABSTRACT

This study is aimed at assessing the effects of *Ficus asperifolia* plant leaf extract on diabetic-induced albino rats. Diabetic animals were treated with various extract doses of *F. asperifolia* leaves for 24 days. The diabetic rats showed a decreased level of blood glucose when compared to rats not treated. There was a significant ($p<0.05$) decrease in serum triglyceride, cholesterol and LDL but a remarkable increase in HDL values of the treated rats compared to the controls administered with distilled water. Haematological analysis also showed that there was a significant increase in PCV, RBC, and Hb values of the treated rats compared to the normal. These results suggested that oral administration of F. asperifolia possess significant anti-diabetic potentials. With this finding it can be concluded that the anti-diabetic effect of *F. asperifolia* may be due to its bioactive compounds responsible for anti-diabetic activity present in the leaf extract.

Key words: Diabetes mellitus, induction, *Ficus asperifolia*, allozan monohdrate, haemetotology, glucose,

INTRODUCTION

Diabetes mellitus is the third leading cause of death (after heart disease and cancer) in many developed countries (Satyarayana and Chakrapani, 2007). This disease condition is caused by abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Das *et al*., 2004). Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Globally, there is an increase in the incidence and prevalence of type 2 diabetes. It was estimated in the year 2000 by the World Health Organization that 171 million people had diabetes, representing 2.8% of the world's population at the time, and also predicted that this number will increase to 366 million (4.4%) by 2030 (Wild *et al*.,2000).

The herbal drugs with anti- diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicines (Magdum *et al.,* 2008).

The physiological activities of plants are attributed to the variety of chemical substances synthesized by plants. These bioactive agents of plants include phytosteroid, alkaloids, saponins, tannins, flavonoids, glycosides, anthraquinones, among many others (Stafford *et al.,* 2004).

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Traditional medicines (herbal) are used for the treatment of diabetes in developing countries where the cost of conventional medicine is a burden to the population (Al, 2010). Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and it's secondary complications continue to be a major medical problem. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess antidiabetic potential (Alarcon *et al.,* 1998).

This rather grim picture is further heightened by the ever soaring high rate of diabetes, in which many of these victims cannot afford medical treatment. It is in this regard that an alternative is being sought in the use of plant as a means of remedy.

Ficus asperifolia belong to the family of *Moraceae* (Adjanahoun*et al*., 1996). Nigerian forests are replete with over 45 different species of Ficus (Keay *et al*., 1964) some of these are *Ficus goliath, Ficus capensis, Ficus carica, Ficus elastica, Ficus exasperata*etc. *F. asperifolia (*Sand paper tree) is commonly known as *Ogbai ikolo* in (Igala) and *Ipin* in Yoruba of ethnic group of Nigeria. It is a small or average size tree, terrestrial or epiphyte which can reach 20m in height. It is one of the many highly medicinal plants with a variety of uses which include its use as anti-tumors, anti-cancer, diuretic, abortifacient, ecobolics and menstrual cycle/ general pain reliever. There are claims that it is also used for the management of diabetes by the Igala tribe of Kogi State, Nigeria. *F. asperifolia* is found across African countries like Senegal,Uganda, Tanzania, Natal (South Africa), Madagascar and Cameroon (Adjanohoun *et al.,* 1996). It is also reported to be found in Michika, Hong and Song Local Government Areas of Adamawa State (Adjanahoun *et al*., 1996), Toro Local Government Area of Bauchi State and Omala Local Government Area of Kogi State all in Northern Nigeria. The aqueous stem extract of *F. asperifolia* is reported to possess hypoglycemic and hypolipidemic properties on diabetic rats but significantly raised serum transaminases activities. It has also been published that the leaf of *F. asperifolia* contained a higher protein, crude fibre and Mineral content than most Nigerian vegetables (Adjanahoun *et al*., 1996). Phytochemical screening of the aqueous leaf extract of *F. asperifolia* have detected the presence of alkaloids, saponins, tannins, cardiac glycosides, terpenes, steroids, balsam and phenol (Rai and Obayemi 1973).

Results from present investigation shows that *Ficus asperifolia* is rich in phytochemicals. Specific biologically important compounds have been identified in extracts from the plant by previous workers (Holets *et al.,* 2003). *F. asperifolia* possess good antioxidant potential presumably because of its phytochemical constituents (Halliwell & Gutteridge, 1992).1, 1-diphenyl-1, 2-picryhydrazyl (DPPH) scavenging activities of *F. asperifolia* showed a good correlation with its reductive potential. These facts justify the medicinal use of the plant for the treatment of various maladies (Dhawan *et al.,* 1977).This study is to assess the potential of *F.asperifolia* for treatment of diabetes mellitus as been postulated in our locality.

MATERIAL AND METHODS

Sources of Plant

The plant was obtained from the premises of Kogi State University Campus Anyigba, & Egume village in Dekina Local government, Kogi State.

Animal sources

The albino rats (*Rattus novergicus*) of weight between 100-200 grams were obtained from Faculty of Medicine, Kogi State University Anyigba.

Methods

Plant preparation:

The leaves were washed with distilled water to remove dirt and impurities. It was air dried at room temperature in the laboratory for four weeks to ensure proper air- drying. The dried leaves samples were pulverized with mortar and pestle. A fine powder was obtained using a mortised blender and sieving. The pulverized leave samples were then stored under dried condition before use.

Plant extraction

The plant extract was done according to Sanni et al,2016.Exactly 200g of powdered *Ficus asperifolia* leaves was weighed using an analytical weighing

Table-1. Plant leaf Extract administration: showing the Oral administration of aqueous extract of *F. asperifolia*

Group	No of rats	Description	Treatment plus normal feeding
A_1	6	Not induced with Diabetes	400mg of plant extract/kg of body weight
A_2	6	Not induced with Diabetes	800mg of plant extract/kg of body weight
B_1	6	Not induced with Diabetes	Water only
B_2	6	Induced with Diabetes	Water only
C	6	Induced with Diabetes	400mg of extract/kg of body weight
D	6	Induced with Diabetes	800mg of extract/kg of body weight

balance (PB 3002-5) into a beaker containing 2000ml of boiling water(100 ^{0}C). The mixture was stirred with a glass rod, covered with a foil paper and then left for 24 hours. Vacuum filtration was carried out after 24 hours using vacuum pump. The filtrate was evaporated in water bath set at 80^{0}C. The concentrate was then stored in the refrigerator prior to use.

Experimental design

The animals selected for this experiment were the healthy albino rats with body weight ranging from 100-200 grams. A total of 36 rats (18 induced diabetic rats, 18 normal rats) were used. Both the induced diabetic and normal rats were randomly selected. These rats were divided into 6 groups of six rats per group, they were all fed with the normal rat feed obtained from Top Feed, Anyigba, Kogi State Nigeria.

Diabetes Induction

A single 120mg/kg of body weight dose of alloxan monohydrate in saline solution was administered intraperitoneally after having fast the animals for 12hours. The animals were returned to their cages after injection and allowed free access to food and water. The blood glucose level were checked after four days from blood taken from their tail by One Touch Ultra glucometer. Animals with blood glucose concentration above 180mg/dl were used for this research work.

Experimental Analysis

The fasting blood glucose level of the rats as well as their weight is been monitored every 4 days for the twenty four days of treatment. The rats were sacrificed on the last day.

Blood Sample Collection.

Blood samples for glucose level determination were collected from the tail tip of the rats. Blood was obtained for haematology and liver function indices analysis. The rats were anaesthetized in a jar containing cotton wool soaked in diethyl ether, the blood was collected via jugular puncture and in plain and EDTA bottle for haematological parameters and liver function indices. The one in plain bottle was allowed to stand for 10 minutes to clot; the serum was then collected using a Pasteur pipette after centrifuge for 10minutes at 1000rpm. The whole blood collected in EDTA bottle was then used for haematological parameters.

Blood Sample Analysis

Haematological analysis

The haematological parameters packed cell volume (PCV (L/L)), haemoglobin Hb(g/L), RBC (X 10^{12} (cell/L),lymphocytes, neutrophils, mean corpuscular volume (MCV (fL)), and mean corpuscular haemoglobin concentration (MCHC (g/L)) and MCH(Pg) were analysed by Automated Haemology Analyzer (SYSMEX KOX1: Sysmex corporation, Japan).

Lipid Profile Analysis

The lipid profile assays were performed using reagent kits supplied by LABKIT. The assay was carried out on the serum.

Triglyceride concentration

The triglycerides were determined by glycerol phosphate dehydrogenase-peroxidase (GPH-POD) enzymatic colorimetric reaction, according to the method described by Buccolo *et al., (*1982).

Serum Cholesterol determination

The total serum cholesterol was determined by the method described by (Allain et al, 1974).

Statistical Analysis

These data were subjected to statistical analysis using a one-way Analysis of Variance (ANOVA) by Graph pad Instant Software.

RESULTS

The result shows that there is no significant difference (p<0.05) between the weight of the rats before induction. However, there was a significant difference between the rats induced with diabetes and the experimental controls during treatment with the plant extract

The result shows the level of glucose in the rats induced with diabetes and normal rats with distilled water only. The result showed that there was a significant difference (p<0.05) in the rats administered with plant extract of 800mg and 400mg in glucose level when compared to the rats administered with water only.

The result showed a significant decrease (p<0.05) in triglycerides, cholesterol and LDL. However, statistical analysis showed that there was a significant increase in HDL in all the treated rats.

The result in the table 8 showed that the PCV, Hb, RBC, MCV, MCHC and MCH value were significantly higher (p<0.05) in group A1 and A2 compared to the rest of the groups. However, the rats treated with extract were significantly higher in these values than the rats induced with diabetes but not treated with extract. Also, the result showed that the white blood cells (WBC), lymphocytes, neutrophils level were higher in the rats induced with diabetes and not treated compared to the control group (fed with water only).

DISCUSSION

Table-2. Weight of the Rats before and after induction of Diabetes with Alloxan Monohydrate.

Group rat	Weight before induction (g)	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24
A1	150±0.53	145±5.87^{a}	160±9.70	150±1.75^{a}	142±2.42^{a}	145±6.69^{a}	135±1.49^{a}
A2	154±6.35	149±1.03	165±0.6^{b}	156±1.72^{b}	149±5.95^{b}	148±4.11^{b}	155±5.71^{b}
B1	155±6.05	167±6.18^{ac}	170±0.2^{c}	170±4.34^{ac}	172±7.58^{abc}	173±8.25^{abc}	178±6.20^{abc}
B2	152±1.56	141±1.87^{c}	144±4.1^{bc}	138±1.11^{ac}	138±1.46^{c}	139±2.03^{c}	142±3.37^{c}
C	154±6.61	139±9.62^{c}	146±6.9^{bc}	150±5.06^{bc}	142±2.57^{c}	143±1.81^{c}	141±1.39^{c}
D	151±1.41	145±6.66^{c}	148±6.8^{c}	146±1.97^{bc}	146±1.60^{c}	149±1.04^{c}	149±2.89^{c}

Values are expressed in gram (Mean)± SD; n = 6. Values with the same superscript on the same column are considered significant (p<0.05).
A1= rat not induced with diabetes but administered with 400mg extract/kg body weight
A2 = rat not induced with diabetes but administered with 800mg extract/kg body weight
B1 = rat not induced with diabetes but administered with water
B2 = rat induced with diabetes administered with water
C = rat induced with diabetes but administered with 400mg extract/kg body weight
D = rat induced with diabetes administered with 800mg extract/kg body weight

Table-3. Glucose Level of the rats during Administered with *ficus asperifolia* Plant Leaf Extract

GROUPS	DAY 4	DAY 8	DAY 12	DAY 16	DAY 20	DAY 24
A1	100±0.6^{a}	98±6.0^{a}	95±1.3^{a}	86±1.1^{a}	73±4.7^{a}	66±4.0^{a}
A2	127±2.6^{b}	99±3.1^{b}	82±3.0^{b}	81±2.3^{b}	75±3.5^{b}	72±2.0^{b}
B1	126±2.1^{c}	126±2.1^{c}	126±2.1^{c}	126±1.9^{ce}	128±1.9^{c}	127±1.6^{c}
B2	263±5.1^{abc}	263±5.0^{abc}	264±5.0^{abc}	267±5.0^{abd}	258±5.1^{abcd}	251±5.1^{abcd}
C	287±2.0^{ab}	277±1.4^{abc}	274±1.4^{abc}	270±1.4^{abc}	251±2.3^{abce}	232±2.6^{ac}
D	232±4.0^{abc}	226±7.0^{abc}	207±3.2^{abc}	192±2.3^{abde}	167±1.1^{abde}	166±1.2^{abd}

Glucose concentrations are expressed in mg/dl ± SD; n=6, column values with the same superscript are considered significant (p<0.05). These data were obtained using a one-way Analysis of Variance (ANOVA) in a Graph pad Instant Software.
A1= rat not induced with diabetes but administered with 400mg extract/kg body weight
A2 = rat not induced with diabetes but administered with 800mg extract/kg body weight
B1 = rat not induced with diabetes but administered with water
B2 = rat induced with diabetes administered with water
C = rat induced with diabetes but administered with 400mg extract/kg body weight
D = rat induced with diabetes administered with 800mg extract/kg body weight

The prevalence of diabetes in our world is becoming a common problem and there are secondary complications associated with this disease condition which requires an immediate solution. This study assessed the anti-hyperglycaemic effect of aqueous leaf extract of *F. asperifolia* following experimentally-induced toxic damage to the pancreas leading to the deficiency of insulin. This condition is called the Type-1 diabetes mellitus. This study however, showed the administration of aqueous leaf extract of *F. asperifolia* have led to a significant decrease (p<0.05) in the blood glucose concentration of the alloxan-induced diabetic rats when compared to the experimental controls. This result is in line with the findings of Omoniwa and Luka (2012). This may be attributed to the hypoglycaemic effect of the plant extract or its ability to stimulate the regeneration of dead β-cells as concluded by the experiments carried out by Mohammed*et al.* (2010).

Table-4. Lipid profile parameters of the Rats treated with *Ficus asperifolia* plant leaf extracts.

Groups	Triglyceride	HDL	Cholesterol	LDL
A1	140±1.4	43±5.1^{a}	83±1.9^{a}	14±4.4^{a}
A2	144±4.3	48±3.9^{b}	87±1.9^{b}	21±0.1
B1	143±4.6	34±6.8^{c}	99±1.9^{a}	34±3.9
B2	140±1.0	48±8.5^{d}	99±1.0^{ab}	36±1.0^{a}
C	148±1.5	80±1.7^{abcde}	100±1.2^{ab}	33±0.3
D	144±0.3	133±3.9^{abcde}	89±1.1	23±1.2

Concentrations are expressed in mg/dl (Mean) ± SD; n=6, Column values with the same superscript are considered significant (P<0.05).
A1= rat not induced with diabetes but administered with 400mg extract/kg body weight
A2 = rat not induced with diabetes but administered with 800mg extract/kg body weight
B1 = rat not induced with diabetes but administered with water
B2 = rat induced with diabetes administered with water
C = rat induced with diabetes but administered with 400mg extract/kg body weight

Table-5. Haematological analysis of the rats treated with *Ficus asperifolia* plant leaf extracts.

GROUPS	PCV	Hb	WBC	RBC	MCV	MCHC	NEUTR.	LYMPH.	MCH
A1	45±2.7	15±0.2	5±0.2^{d}	4.8±0.7	88±1.3^{a}	33±0.3	14±4.0^{a}	80±3.6	29±0.4^{a}
A2	50±1.7^{b}	17±1.1^{b}	5±0.3^{b}	5.1±0.3	114±1.1^{abd}	33±0.1	10±3.6^{bd}	72±2.7^{e}	38±0.4^{abd}
B1	44±5.6	15±1.4	5±0.3^{d}	4.7±0.3	113±1.9^{abdef}	33±0.7	19±3.1^{a}	72±2.7^{cde}	37±0.5^{abdef}
B2	37±1.7^{b}	12±0.7^{b}	7±0.4^{bd}	3.9±0.4	79±9.2^{d}	33±0.2	28±3.0^{df}	90±4.6^{cd}	26±0.9^{d}
C	40±3.6	14±0.8^{b}	6±0.4^{b}	4.4±0.6	85±0.6^{be}	33±0.6	28±3.0^{ab}	86±4.0^{e}	29±0.6^{def}
D	42±1.7	14±1.6	6±0.2	4.6±0.2	91±1.3^{bdf}	33±0.3	20±2.0^{f}	81±3.6	30±0.5^{bdf}

PCV (L/L), Hb (g/L), MCH (Pg), MCHC (g/L), MCV (fL). n = 6 Values are expressed in Mean ± SD. Values with the same superscript on the column is considered significant. (p<0.05). These values were obtained using a one-way Analysis of Variance (ANOVA) in a Graph pad Instant Software.
A1= rat not induced with diabetes but administered with 400mg extract/kg body weight
A2 = rat not induced with diabetes but administered with 800mg extract/kg body weight
B1 = rat not induced with diabetes but administered with water
B2 = rat induced with diabetes administered with water
C = rat induced with diabetes but administered with 400mg extract/kg body weight
D = rat induced with diabetes administered with 800mg extract/kg body weight

The results of haematological analysis indicated that treatment with aqueous leaf extract of *F. asperifolia* significantly (p<0.05) elevated all the heamolytic parameters in diabetic-induced rats compare to the controls during treatment. The result showed that the leaf extract of *F. asperifolia* have anti-anaemic potentials. These findings agree with the previous report by Dina *et al.,* 2000. The difference in the anti-anaemic potentials of *F. asperifolia* extracts might be due to the different phytochemicals present especially the polyphenols (Flavanoids) (Dina *et al.,* 2000)

In diabetes mellitus, insulin deficiency causes excessive mobilization of free fatty acids and under-utilization of chylomicrons and VLDL, leading to hypertriacylglycerolaemia. Elevated levels of cholesterol present in LDL are associated with atherosclerosis, whereas high levels of HDL have protective effect (Murray *et al.,* 2003). In this study, diabetic animals that received the various doses of extract had a significantly (p<0.05) reduction in Triglyceride, cholesterol and LDL whereas there was a significant increase (p<0.05) in HDL concentration than the diabetic untreated rats. The result showed that there was uptake of plasma lipids for storage which was made possible by either the insulin-mimetic property of the plant. These findings correlate with the experiment of Omoniwa and Luka (2012).

CONCLUSIONS

The results obtained in this research work shows that the aqueous leaf extract of *F. asperifolia* at the doses tested (400mg/kg b.w and 800mg/kg b.w) possess anti-hyperglycaemic properties and may have beneficial effects in Type-1 diabetes mellitus. It can also be ascertained that the plant extract may serve as a source of bioactive molecules for future generation of anti-diabetic drugs. Comprehensive pharmacological and phytochemical investigation is however required to determine the extract mechanism of the action of the anti-hyperglycaemic activity of the extracts/fraction as well as to isolate and elucidate the structure of the bioactive molecules responsible for the effects observed.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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