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ASSESSMENT OF THE EFFECT OF FICUS EXASPERATA (VAHL) AQUEOUS LEAF EXTRACTS ON BODY WEIGHT AND SERUM LIVER ENZYMES IN HYPERGLYCAEMIC WISTAR RATS

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ABSTRACT

Various pharmacological actions such as anti-diabetic, lipid lowering and antifungal activities have been reported for *Ficus exasperata*. The aim of the present study was to investigate the effect of giving oral administration of *Ficus exasperata* extract with different dosages on body weight and liver function enzymes in streptozotocin-induced diabetic rats and normal rats. Forty – Two adult Wistar rats of both sexes weighing between 200 – 235g were used for this study. The rats were bred in the animal holding of the Department of Anatomy, University of Benin, Benin City. The animals were randomly assigned into seven groups of Six rats each. Group 1 was the Normal Control Group. Group 2 served as Diabetic Control Group without extract treatment. Group 3 was the experimentally induced diabetic rats treated with a standard anti-diabetic drug 5mg/kg of Glibenclamide. Group 4 was the experimentally induced diabetic rats treated with aqueous extracts of 100 mg/kg of *Ficus exasperata*. Group 5 was the experimentally induced diabetic rats treated with aqueous extracts of 200mg/kg of *Ficus exasperata*. Group 6 was the non – diabetic rats treated with aqueous extracts of 100mg/kg of *Ficus exasperata*. Group 7 was the non – diabetic rats treated with aqueous extracts of 200mg/kg of *Ficus exasperata*. Diabetes Mellitus was induced in groups 2, 3, 4, 5 rats by a single intraperitoneal injection of Streptozotocin (STZ) (60 mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.8). The results from this study showed significant weight loss in the group of untreated diabetic animals. Furthermore, the study demonstrated significant increase in the activities of serum Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) of diabetic rats as compared to the control group. Treatment with either aqueous leaf extracts of *Ficus exasperata* or glibenclamide drug produced significant decrease in serum AST, ALT, ALP activities, as compared to the diabetic group. In conclusion, the results of this study showed that oral administration of the crude aqueous leaf extracts of *Ficus exasperata* has the potential to counter the hepatotoxicity presented by streptozotocin induced diabetes mellitus in wistar rats.

INTRODUCTION

Ficus exasperata popularly referred to as "sand paper tree" in Nigeria owing to the rough surface of the leaves, is increasingly being used for a number of ailments, as such, studies on the traditional uses and scientific evaluation are on the increase. Several parts of the plant have been used in traditional medicine for treatment of several pathologies. The genus *Ficus* has about 800 species occurring in the warmer part of the world. Nigeria forests are replete with over 45 different species of Ficus[1]. Some of them are *Ficus* goliath, Ficus capensis, *Ficus ingens*, *Ficus glomosa*, Ficus lecardi and *Ficus elastica*. They can be found in the savanna, rain forest, beside rivers and streams. The leaf extract from *Ficus exasperata* is reported to have diverse uses such as treating hypertensive patients [2], haemostative opthalmia, coughs and haemorrhoids [3]. In Nigeria, young leaves of *Ficus exasperata* are prescribed as a common anti-ulcer remedy. Various pharmacological actions such as anti-diabetic, lipid lowering and antifungal activities have been reported for *Ficus exasperata*[4].

Diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [5]. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs[5]. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. The management of Diabetes mellitus depends on continuous hypoglycaemic therapy.

KEYWORDS: *Ficus Exasperata, Body weight, Liver Function, Diabetic rats*

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MATERIALS AND METHODS

PREPARATION OF AQUEOUS EXTRACT OF *FICUS EXASPERATA* (VAHL)

The Leaves of *Ficus exasperata* were collected from the University of Benin, in Benin City, Nigeria and were subsequently sun dried. This was later powdered in the Pharmacognosy Laboratory of the Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria. This powdered form of the *Ficus exasperata* (Vahl) was weighed using an electronic weighing scale to give 2kg. Two litres of distilled water was used to soak the powdered sample for 24 hours, followed by concentration in water (warm) bath. The extract was then oven dried at 40°c using Gallenkamp oven for another 72h (3days). The weight of the extract was given at 1234.57g. The total percentage yield of the extract was given at 61.72%. Preliminary phytochemical analyses were also carried out.

ANIMAL CARE

Forty – Two adult Wistar rats of both sexes weighing between 200 – 235g were used for this experiment. The rats were bred in the animal holding of the Department of Anatomy, University of Benin, Benin City. They were fed with livestock broiler finishers manufactured by Top-feed limited and were given water ad libitum.

EXPERIMENTAL DESIGN

The animals were randomly assigned into seven groups 1, 2, 3, 4, 5, 6, 7 of Six rats each.

Group 1 was the control (Normal Control Group)

Group 2 was the experimentally induced diabetic rats without *Ficus exasperata* treatment (Diabetic Control Group)

Group 3 was the experimentally induced diabetic rats treated with a standard anti-diabetic drug 5mg/kg of Glibenclamide

Group 4 was the experimentally induced diabetic rats treated with aqueous extracts of 100 mg/kg of *Ficus exasperata*

Group 5 was the experimentally induced diabetic rats treated with aqueous extracts of 200mg/kg of *Ficus exasperata*

Group 6 was the non – diabetic rats treated with aqueous extracts of 100mg/kg of *Ficus exasperata*

Group 7 was the non – diabetic rats treated with aqueous extracts of 200mg/kg of *Ficus exasperata*

INDUCTION OF EXPERIMENTAL DIABETES MELLITUS

Diabetes Mellitus was induced in groups 2, 3, 4, 5 rats by a single intraperitoneal injection of Streptozotocin (STZ) (60 mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.8). Animals in group 1 were given equal volume of citrate buffer used in dissolving streptozotocin intraperitoneally. Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period of 72-hours.

ADMINISTRATION OF *FICUS EXASPERATA* EXTRACTS

Leaf Aqueous Extracts of *Ficus exasperata* was administered at 100mg/kg to rats in groups 4 (diabetic) and 6 (non-diabetic) while 200mg/kg was administered to rats in group 5 (diabetic) and 7 (non-diabetic) daily through orogastric tube for 14 days. A standard antidiabetic drug (glibenclamide 5mg/kg) was administered to group 3 rats for 14 days, while those in group 2 were left untreated.

DETERMINATION OF BODY WEIGHTS

The body weights of the animals were monitored every 72 hours with a weighing balance

SACRIFICE OF ANIMALS

At the end of the experimental period, the animals were grossly observed for general physical characteristics, mobility and agility. They were also screened for the presence of open wounds. The animals were weighed with a weighing balance. A midline incision was made through the anterior abdominal walls of the rats under light anaesthesia using chloroform. Blood samples were collected, transferred to sterilized tubes and allowed to clot at room temperature. The blood samples were centrifuged in a Denley BS400 centrifuge (England) for 5 minutes at 5000 rpm to obtain serum. Serum obtained was used for estimation of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and Total Protein (TP).

STATISTICAL ANALYSIS

The data were analysed using descriptive and inferential statistics. All values were presented as mean $\pm$ standard error of mean (SEM) for six rats each of seven groups. The significance of difference in the means of all parameters was determined using one way analysis of variance (ANOVA; 95% confidence interval). Least Square difference, post hoc tests was carried out for all groups with control and comparison of all pairs of groups respectively. All statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) (version 17).

RESULTS

EFFECTS OF *FICUS EXASPERATA* ON THE BODY WEIGHT

The animals in the control group showed significant ($P < 0.05$) increase (212.5 ± 3.8; 232.5 ± 3.6) in body weight (figure 1) while diabetic animals in group 2 that were not treated with extract showed significant ($P < 0.05$) decrease (211.7 ± 4.2; 188.3 ± 2.8) in body weight (figure 2). Figure 3 shows no significant ($P > 0.05$) difference (213.3 ± 4.4; 203.3 ± 2.5) in body weight of animals in group 3 (diabetic rats treated with 5mg/kg body weight (bw) glibenclamide). Bar chart in figures 4&5 shows the effect of aqueous

extract of *Ficus exasperata* on diabetic animals. Group 4 (diabetic animals treated with 100mg/kg bw of *Ficus exasperata*) showed no significant (P>0.05) difference (212.5±5.6; 213.3±3.8) in body weight while diabetic animals that received 200mg/kg bw of *Ficus exasperata* extract (group 5) showed significant (P<0.05) decrease (212.5±5.7; 205.8±3.3) in body weight. Bar chart in Figures 6&7 shows the effect of aqueous extract of Ficus exasperata on non-diabetic animals. Group 6 (non-diabetic animals treated with 100mg/kg bw of *Ficus exasperata*) and group 7 (non-diabetic animals treated with 200mg/kg bw of *Ficus exasperata*) showed significant (P<0.05) increase in body weight (213.3±2.8; 224.2±3.3 and 213.3±2.1; 223.3±4.0) respectively.

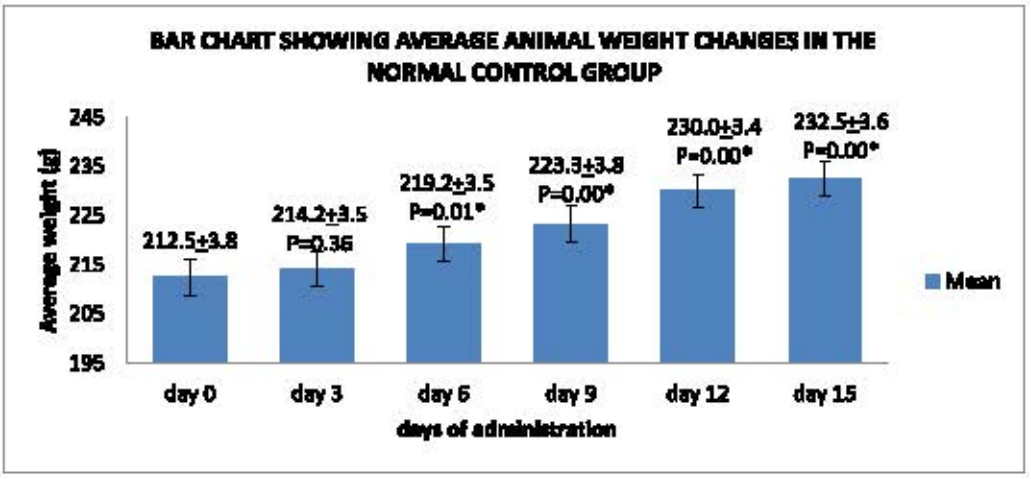

Figure 1 : BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE NORMAL CONTROL GROUP 1

***Significant difference at (p <0.05)**

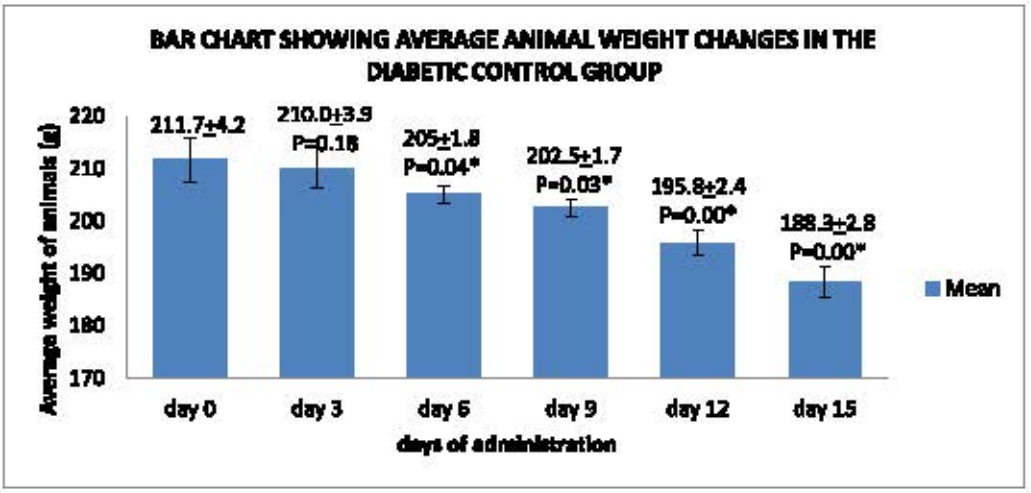

FIGURE 2: BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE DIABETIC CONTROL GROUP 2

***Significant difference at (p <0.05)**

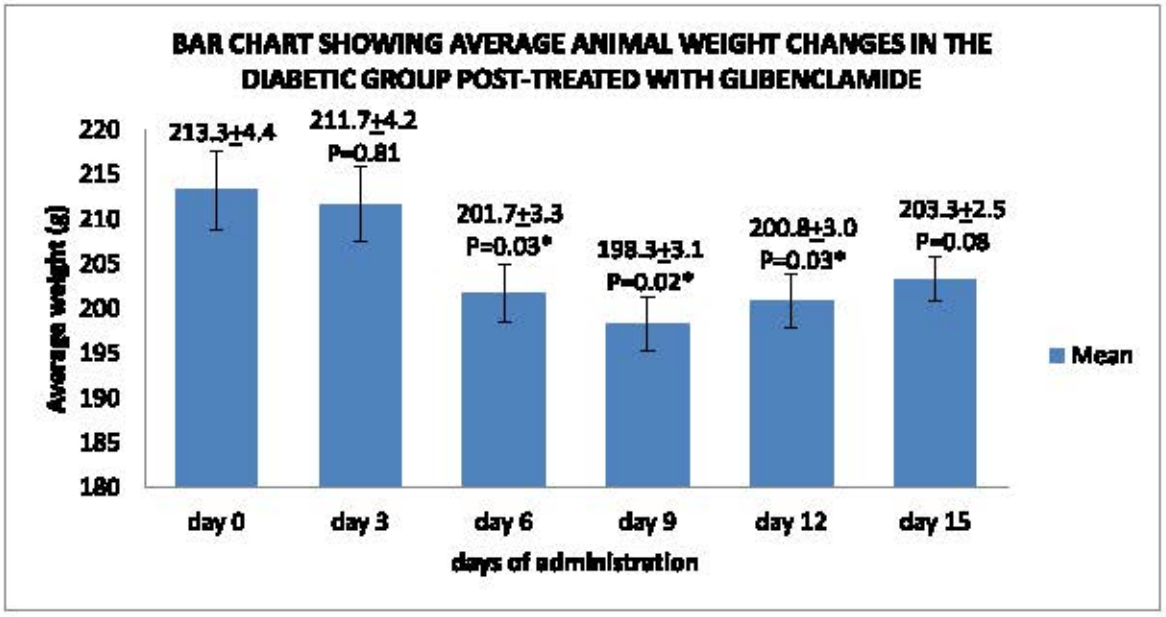

FIGURE 3: BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE DIABETIC GROUP 3 POST-TREATED WITH GLIBENCLAMIDE

*Significant difference at ($p < 0.05$)

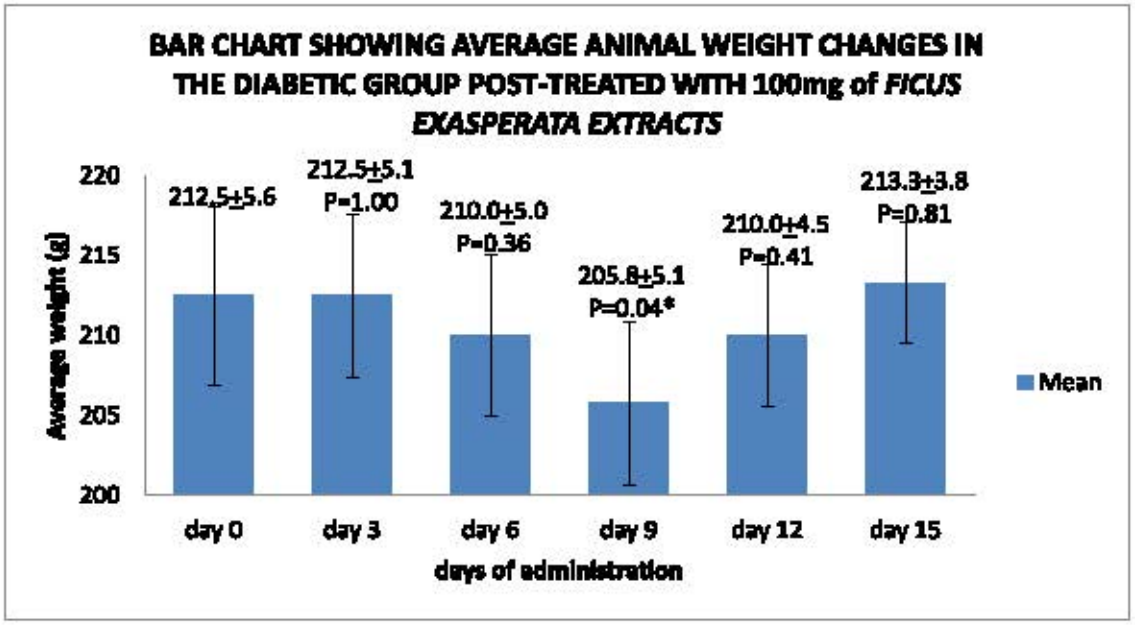

FIGURE 4 : BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE DIABETIC GROUP 4 POST-TREATED WITH 100mg of *FICUS EXASPERATA EXTRACTS*

*Significant difference at ($p < 0.05$)

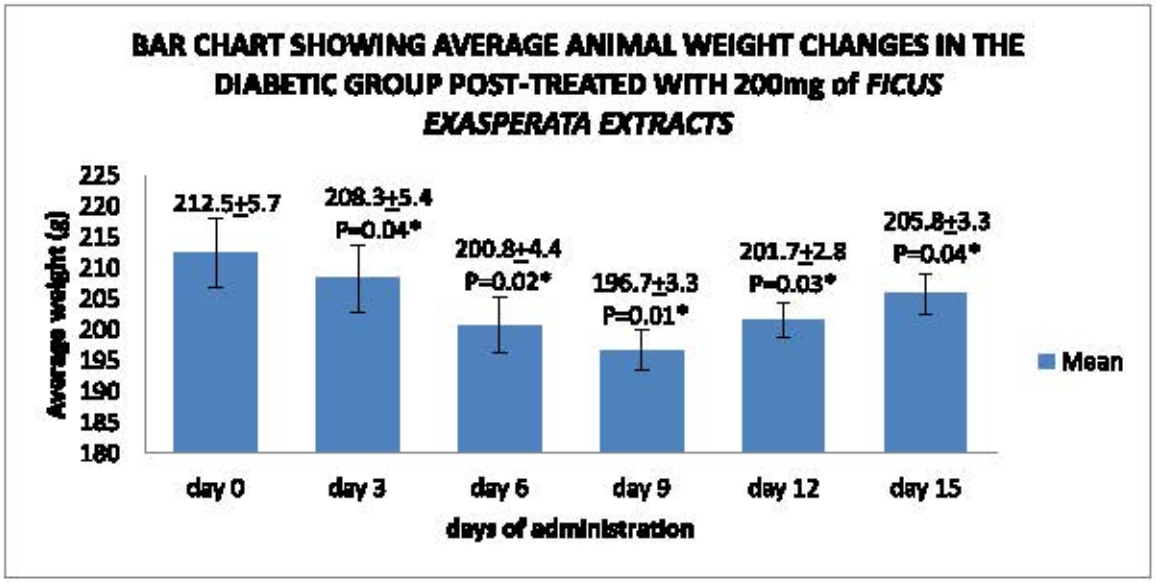

FIGURE 5: BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE DIABETIC GROUP 5 POST-TREATED WITH 200mg of *FICUS EXASPERATA EXTRACTS*

*Significant difference at ($p < 0.05$)

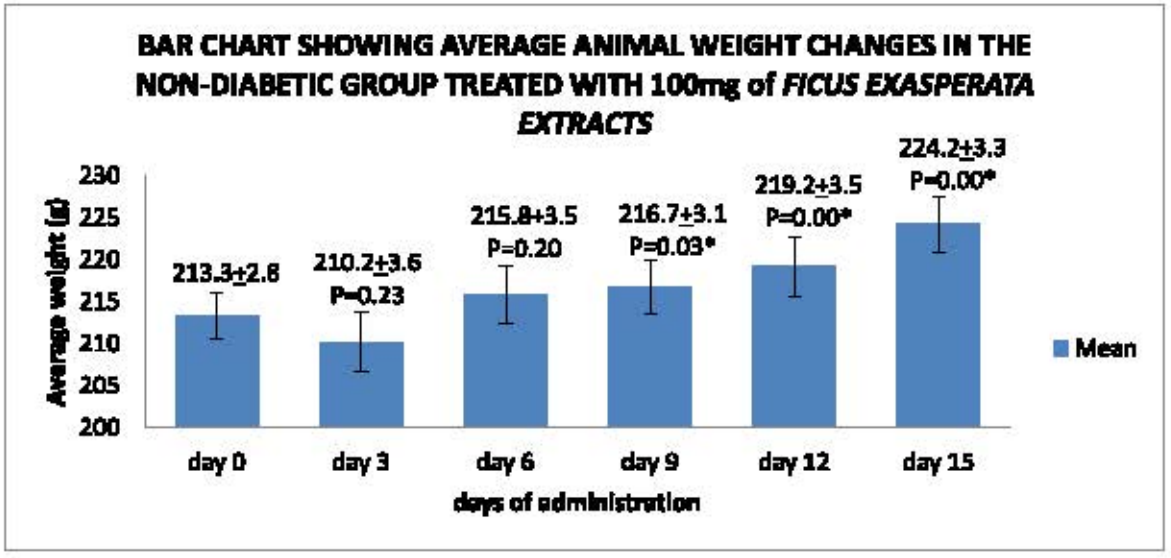

FIGURE 6: BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE NON-DIABETIC GROUP 6 TREATED WITH 100mg of *FICUS EXASPERATA EXTRACTS*

*Significant difference at ($p < 0.05$)

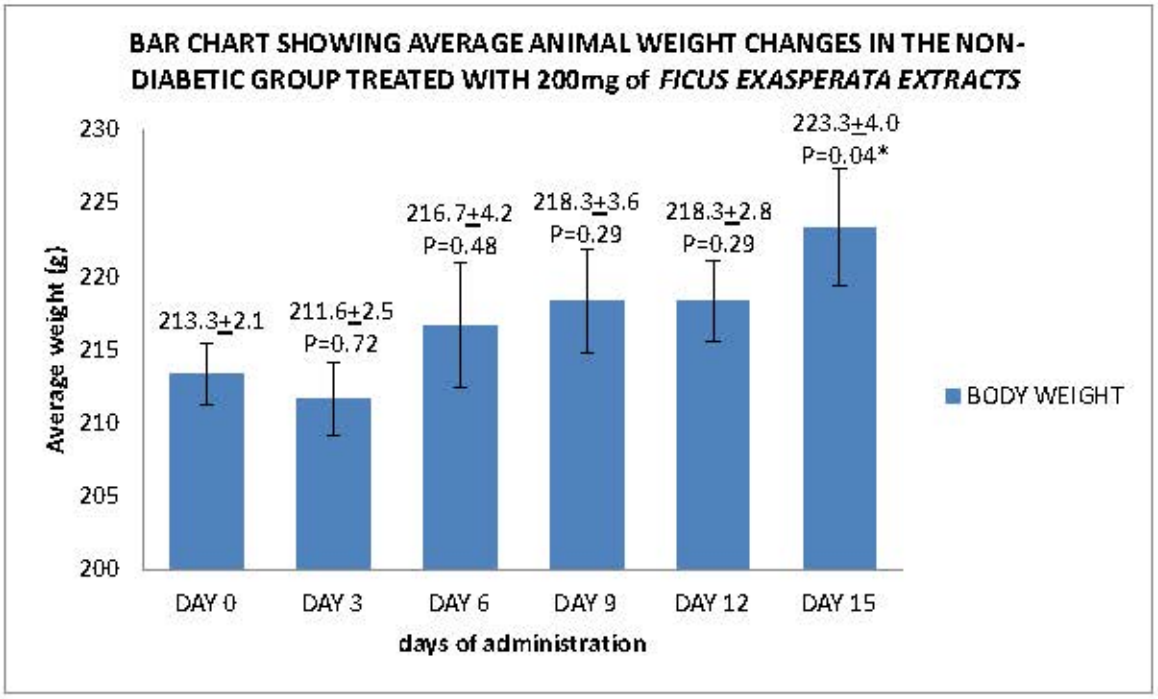

FIGURE 7: BAR CHART SHOWING AVERAGE ANIMAL WEIGHT CHANGES IN THE NON-DIABETIC GROUP 7 TREATED WITH 200mg of *FICUS EXASPERATA EXTRACTS*

*Significant difference at ($p < 0.05$)

EFFECTS OF *FICUS EXASPERATA* ON LIVER FUNCTION ENZYMES AND SERUM TOTAL PROTEIN

Table 1 illustrates the effects of *Ficus exasperata* on the activity of Total Protein (TP), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatase (ALP) respectively in the serum of experimental groups 1-7. The activity of AST, ALT and ALP significantly increased ($p < 0.05$) in the untreated streptozotocin (STZ) induced diabetic rats when compared to the control group of rats. The activity of these liver enzymes is significantly lowered in the *Ficus exasperata* treated diabetic group. However *Ficus exasperata* has no effect on the activity of these enzymes in normal rats. Furthermore, the activity of total protein is significantly ($p < 0.05$) reduced in the untreated diabetic rats when compared to the control and treated diabetic rat groups. *Ficus exasperata* treatment on non diabetic rats in group 6 and 7 showed no significant difference when compared with the control group 1

Groups	Group 1 (control)	Group 2 (STZ only)	Group 3 (STZ + glibenclamide)	Group 4 (STZ + *Ficus* 100mg/kg)	Group 5 (STZ + *Ficus* 200mg/kg)	Group 6 (*Ficus* 100mg/kg only)	Group 7 (*Ficus* 200mg/kg only)
AST (IU/L)	23.5 ± 0.43	40.50±0.43 [a]	35.50±0.43 [a, b]	28.00±0.58 [b]	34.00±0.58 [a, b]	24.67±0.71[b]	25.83±1.01 [b]
ALT (IU/L)	25.50±0.43	43.00±0.58 [a]	37.33±0.42 [a, b]	31.50±0.43 [b]	34.17±1.17[b]	26.33±0.67 [b]	28.67±1.36 [b]
ALP (IU/L)	73.33±2.47	143.33±1.5[a]	102.50±4.81[a, b]	84.67±1.43[b]	99.50±3.30[b]	79.00±1.46[b]	81.83±1.22[b]
TOTAL PROTEIN (mg/dl)	8.17±0.31	4.83±0.31[a]	5.67±0.33 [a]	6.33±0.33 [b]	5.93±0.34 [a]	7.53±0.24 [b]	7.00±0.37 [b]

[a]Significantly different from control group; [b]Significantly different from STZ-induced diabetic group; Significant difference at ($p < 0.05$)

DISCUSSION

The present study aimed to investigate the possible effect of the crude aqueous leaf extracts of *Ficus exasperata* on the body weights and liver function enzymes in diabetic and non diabetic adult wistar rats. The results from this study showed significant weight loss in the group of untreated diabetic animals. Granner[7] earlier reported that the loss in weight may be due to the loss in adipose tissue and muscle which results from excessive fatty acid and tissue protein breakdown. One of the symptoms present in diabetes mellitus is weight loss which occurs when there is poor glycaemic control. Ahmed[8] had earlier reported significant weight loss/reduction in untreated diabetic rats. Furthermore, insulin is involved in protein metabolism and stimulates the influx of amino acids into skeletal muscles to increase protein synthesis. In animals with induced diabetes, the decline in such actions of insulin leads to the decline in cellular glucose utilization and a starvation state[9]. This may be the reason why all the diabetic groups had greater weight reductions than the normal group. The observation that the control group showed significant weight gain whereas there was neither significant weight loss or gain in the treated diabetic group agrees with the report of Makimattila[10] that improved glycaemic control by insulin promotes weight gain by decreasing both metabolic rate and glycosuria. Such severe weight loss was prevented in the extract treated groups. However, after induction of diabetes mellitus by streptozotocin, subsequent treatment of diabetic animals with plant extracts and glibenclamide showed appreciable increase in weight. This appreciation in weight indicates that the treatment allowed the tissues to access the glucose both to supply energy and spare some to build tissue materials required for growth. Studies have shown that treatment with extracts of *Flacourtia indica* resulted in appreciation in weight of the animals after 28 days[11]. The results of the phytochemical screening of the crude aqueous leaf extracts of *Ficus exasperata* showed that the samples contained some bioactive substances. Only reducing sugars, tannins and anthraquinons were absent. The values of bioactive substances present in *Ficus exasperata* such as carbohydrate, alkaloids, saponins, flavonoids, cardiac glycosides, steroids and terpenoids were closely related to those already reported

[12], [13]. Literature has equally shown the biological activities of alkaloids and flavonoids to include hypoglycemia, hypolipidemia, hypoazotemia, hypotension among other biological effects[14]. Flavonoids [15]and saponins[16] are well known for their antioxidant and hepatoprotective activities. The presence of these two active biological principles in high concentrations in *Ficus exasperata* leaf extract may be responsible for the hepatoprotective effects recorded in the present study. Aminotransferases (AST and ALT) mediate the catalysis of amino transfer reactions and they are markers for clinical diagnosis of liver injury. Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate group from nucleotides and proteins, it is produced primarily in liver and brain[17] and it is a marker of hepatic functions[18]. This study demonstrated significant increase in the activities of serum AST, ALT, ALP of diabetic rats as compared to the control group. This increase as earlier reported[19] may be due to the leakage of these enzymes from the liver cytosol into the blood stream, which gives an indication of the hepatotoxic effect of Streptozotocin. Treatment with either aqueous leaf extracts of *Ficus exasperata* or glibenclamide drug produced significant decrease in serum AST, ALT, ALP activities, as compared to the diabetic group. The crude aqueous leaf extracts of *Ficus exasperata* is more effective than glibenclamide drug in lowering these serum activities.

In conclusion, the results of this study show that the crude aqueous leaf extracts of *Ficus exasperata* when administered orally has the potential to counter the hepatotoxicity presented by streptozotocin induced diabetes mellitus in adult wistar rats.

REFERENCES

1. Keay RW, Onochie CF. Nigeria Trees. (1964). Vol I & II. Department of Forestry Research, Ibadan.

2. Buniyamin AA, Eric KI, Fabian CA. Pharmacognosy and Hypotensive evaluation of Ficus exasperata Vahl (moraceae) leave. Acta Poloniae Pharmaceutica-Drug Res. (2007). 64(6): 543-546.

3. Odunbaku OA, Ilusanya OA, Akasoro KS. Antibacterial activity of ethanolic leaf extract of Ficus exasperata on Escherichia coli and Staphylococcus albus. Sci. Res. Essay. (2008). 3(11): 562-564.

4. Sonibare MO, Isiaka AO, Taruka MW, Williams NS, Soladoye M, Emmanuel O. Constituents of Ficus exasperata leaves. Natural product communications. (2006). pp. 23-26

5. Alberti KG, Aschner P, Assal JP, Bennett PH, Groop L, Jervell J, Kanazawa Y, Keen H, Klein R, Mbanya JC, McCarty D, Motala A, Pan XR, Ramachandran A, Samad N, Unwin N, Vardi P. Zimmet PZ. Report of a WHO Consultation on Diagnosis and Classification of Diabetes Mellitus. World Health Organization. (1999).

6. Gavin JR, Alberti KG, Davidson MB, DeFronzo RA, Drash A, Gabbe S.G, Genuth S, Harris MI, Kahn R, Keen H, Knowler WC, Lebovitz H, Maclaren NK, Palmer JP, Raskin P, Rizza RA, Stern MP. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. (1997). 20:1183-1197

7. Granner DK. Hormones of the pancreas and gastrointestinal tract. In D. K. Granner (Ed.), Harper's biochemistry 25th ed. (1998). New York: McGraw Hill., 610-626

8. Ahmed SM, Vrushabendra SB, Gopkumar P, Dhanapal R, Chandrashekara J. Antidiabetic acivity of Terminalia catappa Linn. leaf extracts in alloxan-induced diabetic rats. Iranian J. Pharmacol. & Ther. (2005). 4(1), 38-39.

9. Park HR, Cho JS. Effect of natural medicinal plants extracts on organ weight and blood glucose challenge in Streptozotocin-induced diabetic rats. J. Nat. Sci; (2006). 25: 43-53.

10. Makimattila S, Nikkila K, Yki –Jarvinen H. Causes of weight gain during insulin therapy with and without metformin in patients with type II diabetes mellitus. Diabetologia. (1999). 42(4), 406-412.

11. Singh V, Mahendra S, Smita S, Sunil S. Antidiabetic Effect of Flacourtia indica Merr in Streptozotocin Induced Diabetic Rats. Global J. Pharmacol. (2011). 5 (3): 147 - 152

12. Adebayo EA, Ishola OR. Phytochemical and antimicrobial screening of crude extracts from the root, stem bark and leaves of Bridelia ferruginea. Afr. J. Biotech. (2009). 8(4): 650-653.

13. Mensah JK. Okoli RI. Ohaju-Obodo JO. Eifediyi K. Phytochemical, nutritional and medicinal properties of some leafy vegetables consumed by Edo people in Nigeria. Afr. J. Biotechnol. (2008). 7:2304-2309

14. Oladele SB. Ayo JO. Adaudi AO. Medicinal and physiological properties of flavonoids, coumarin derivatives and anthraquinones of plant origin. West Afr. J. Pharmacol. Drug Res. (1995). 11: 134-144.

15. Baek NL. Kim YS. Park, KH. Isolation of antihepatotoxic agent from the roots of Astragalus membranaceous. Korean J Phamacol. (1996). 27: 111-117

16. Yoshikawa M. Morikawa T. Kashima Y. Matsuda H. Structure of new dammaranetype triterpene saponins from the flower buds of Panaxnoto ginseng and hepatoprotective effect of principal Ginseng saponins. J. Nat. Prod. (2003). 66(7): 922-927

17. Han KH, Hashimoto N, Shimada K, Sekikawa M, Noda T, Yamauchi H, Hashimoto M, Chiji H, Topping DL, Fukushima M. Hepatoprotective effects of purple potato extract against Dgalactosamine- induced liver injury in rats. Biosci. Biotechnol. Biochem., (2006). 70: 1432-1437.

18. Yoo YM, Nam JH, Kim MY, Choi J, Park HJ. Pectolinarin and pectolinarigenin of Cirsiumsetidens prevent the hepatic injury in rats caused by D-galactosamine via an antioxidant mechanism. Biol. Pharm. Bull. (2008) 31(4): 760-764.

19. Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM. Free radicals scavenger and antihepatotoxic activity of Rosmarinus. Plant Med. (1993). 59: 312-314.