www.ajbrui.net

Afr. J. Biomed. Res. Vol.18 (January, 2015); 61- 67

Full Length Research Paper

Changes in Biochemical Markers of Kidney Function and Antioxidant Status of Diabetic Rats treated with Aqueous Leaf Extracts of *Ficus exasperata* (Vahl)

Enogieru A.B*, Momodu O.I, Omoruyi S.I, Om'iniabohs F.A.E

[1]Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria

ABSTRACT

Cases of diabetic kidney disease continue to increase worldwide despite advances in knowledge of the disease. Oxidative stress has been shown to play major role in the pathogenesis of diabetes mellitus, since free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and subsequently, oxidative degradation of glycated proteins. This study was designed to investigate the ameliorative and antioxidant effects of crude Aqueous Leaf Extract of *Ficus exasperata* (ALEFE) on the kidney of STZ induced diabetic wistar rats. The investigation involved five (5) groups (A-E) of rats containing six rats (n=6). Group A served as normal control while groups B, C, D and E were injected with STZ 60 mg/kg body weight (bw) intraperitoneally to induce diabetes. Rats in group B served as diabetic control. Rats in groups C, D and E were treated with 5mg/kg bw of glibenclamide orally, 100mg/kg bw of ALEFE and 200mg/kg bw of ALEFE respectively via orogastric tube. Antioxidant results showed significantly increased levels of superoxide dismutase (SOD) and catalase (CAT) in ALEFE treated diabetic rats as compared to untreated diabetic rats. Malonyldialdehyde (MDA) levels were also found to be significantly reduced in ALEFE treated diabetic rats as compared to untreated diabetic rats. Histological findings shows that ALEFE had a protective effect on kidney against STZ toxicity. These findings proffer preliminary biochemical and histological support to the ethno medicinal uses of the plant in the management and/or control of diabetes mellitus.

Keywords: *Ficus exasperata*: Histological: Biochemical: Diabetes: Wistar rats

INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder affecting carbohydrate, fats and protein metabolism, is a major degenerative disease in the world today (Ogbonnia *et al.*, 2008). Its worldwide prevalence is estimated to be between 1% and 5% of the world population (Kameswararao *et al.*, 2003; Petal and Rybczynski, 2003). It is considered to be at epidemic level by the World Health Organisation (Petal and Rybczynski, 2003). In 2000, according to the World Health Organisation (WHO), at least 171 million people worldwide suffered from diabetes, or 2.8% of the population (Wild *et al.*, 2004). Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double (Wild *et al.*, 2004). The Diabetes Association of Nigeria (DAN) had put the diabetic's population in Nigeria at about 10 Million as at 2004 (Ogbera *et al.*, 2005). Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Abnormally elevated blood glucose level causes oxidative stress and the

*Corresponding author:
E-mail: bizou.enogieru@uniben.edu
Tel: +2347016780198

Date Received: May 2014, Date Accepted:, October, 2014

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

formation of advanced glycation end products which result in diabetic complications (Ahmed, 2005).

Among the complications of diabetes, nephropathy seems to be prevalent (Sawiress, 2011). Clinical trials suggest that there is no effective treatment for diabetic nephropathy, thus efforts are focusing on traditional herbal medicine to find a novel therapeutic agents for treatment of diabetic nephropathy (Kang et al., 2006).

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. 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. Ijeh *et al.*, 2007 reported increase in body weight, serum urea and sodium concentration following administration of ethanol extracts of *Ficus exasperata* at 50-500mg/kg body weight in three days.

The aim of the present study therefore, is to evaluate the possible beneficial effect of *Ficus exasperata* on diabetic nephropathy in Streptozotocin-induced diabetic Wistar rats using biochemical and histological means.

MATERIALS AND METHOS

Animals and Animal Handling

Thirty (30) adult rats of the Wistar strain weighing between 200g – 235g of both sexes were used. They were fed with livestock broiler finishers manufactured by Top-Feed Limited and were given water *ad libitum.* The Leaves of *Ficus exasperata* were collected from the environment of the University of Benin, in Benin City, Nigeria. The leaves were authenticated in the department of Botany, University of Benin, Benin City. They were subsequently sun dried and processed in the Department of Pharmacognosy Laboratory of the Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria.

Induction of Diabetes

Diabetes Mellitus was induced in groups B, C, D and E 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 A 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. However before the induction of diabetes, all the animals were fasted for 16-h, but still allowed free access to water. At the end of the 16-h fasting period – taken as 0 time (i.e., 0 h) – The blood glucose levels (initial glycaemia, G_0) of the fasted (control), and other experimental rats were determined and recorded. All the animals were kept and maintained under laboratory conditions of light, humidity and temperature.

Experimental Design

The animals were randomly assigned into five groups A-E of Six rats each.

- Group A were the control (Normal Control Group)
- Group B were the experimentally induced diabetic rats without *Ficus exasperata* treatment (Diabetic Control Group)
- Group C were the experimentally induced diabetic rats treated with a standard anti-diabetic drug 5mg/kg of Glibenclamide
- Group D were the experimentally induced diabetic rats treated with 100 mg ALEFE
- Group E were the experimentally induced diabetic rats treated with 200mg ALEFE

Biochemical parameters

On the 15th day of the study, blood samples were collected after sacrifice of animals and used for determination of serum creatinine, urea and electrolyte levels. Rats were scarified by cervical dislocation. After weighing, one ipsilateral kidney was removed, washed with physiological saline, cleared of fatty tissue and weighed. They were homogenized in ice cold 20 mM Tris-HCl buffer (pH 7.4) and the homogenates were then centrifuged at 10,000 g for 10 min at 4ºC (Montilla *et al.*, 2005). The supernatants were collected and used for assessment of Superoxide Dismutase (Sun *et al.*, 1998), Catalase (Aebi, 1984) and Malonyldialdehyde (Deniz *et al.*, 1997).

Tissue Processing/Photomicrography

The second of the harvested kidneys was fixed in formal saline and then processed for microscopy using the Haematoxylin And Eosin Staining Method Of Drury, Wallington And Cameron (1976).

Determination of Relative Kidney Weight

At sacrifice, the weight of the kidney was determined using a top loader sensitive balance (Mettler Toledo, Germany). To reduce the individual body weight differences, the relative organ weight (%) was calculated from the body weight at sacrifice and the absolute weights of each organ (Kim et. al., 2008) as follows:

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrifice}} \times 100$$

Statistical Analysis

The data were analysed using descriptive and inferential statistics. All values were presented as mean

± standard error of mean (SEM) for six rats each for the five groups. The significance of difference in the means of all parameters was determined using one way analysis of variance (ANOVA) at 95% confidence interval. Least Square difference, *post hoc* tests were carried out for all groups with control and comparison of all pairs of groups respectively.

RESULTS

Effect of *Ficus exasperata* on blood glucose concentrations

The profile of changes in blood glucose levels of the rats before, during and after treatment with ALEFE is as shown in Table 1. At Day 0 (72 hours after STZ administration), the fasting blood glucose level differed significantly ($p < 0.05$) between the diabetic and control groups as it was significantly higher in the diabetic groups (B, C, D, E). At the end of the experiment (Day 15), blood glucose levels of rats in group C, D and E were significantly reduced on treatment with Glibenclamide and *Ficus exasperata* respectively.

Effects of *Ficus Exasperata* on relative kidney weight

The relative kidney weight of untreated diabetic rats was significantly ($p< 0.05$) low (0.338 ± 0.007 %) when compared with the control normal rats (0.388 ± 0.08 %). Glibenclamide and *Ficus exasperata* treated diabetic rats showed no significant ($p > 0.05$) difference when compared with normal control rats of group A (Fig. 1).

Effects of *Ficus exasperata* on kidney function Enzymes and Electrolytes

Table 2 shows the blood glucose levels of the animals in each group before and after induction of experimental diabetes. It also shows the effects of *Ficus exasperata* on the concentration of creatinine, urea and electrolyte levels in the serum of STZ induced diabetic rats. The concentration of creatinine and urea in the serum of STZ induced diabetic rats (1.82 ± 0.10 mg/dl and 100.67 ± 6.00) respectively is significantly high when compared with that of the control group of rats (0.72 ± 0.11 mg/dl and 38.83 ± 4.37 mg/dl) respectively. Urea levels were significantly ($P < 0.05$) lowered in rats treated with 100 mg/kg of *Ficus exasperata* but not in rats treated with glibenclamide and 200 mg/kg. Creatinine levels were significantly ($P < 0.05$) lowered in rats treated with 100 mg/kg of *Ficus exasperata* but not in rats treated with glibenclamide and 200 mg/kg of *Ficus exasperata*. Treatment groups showed varying levels of significance in electrolyte markers.

Effects of *Ficus exasperata* on kidney tissue Antioxidant Enzymes

Table 3 shows the effects of *Ficus exasperata* on the activity of Catalase (CAT) and superoxide dismutase (SOD) in the kidney of experimental groups A-E. The activity of CAT and SOD in the kidney of STZ induced diabetic rats (3.5 ± 0.43 U/mg and 13.17 ± 0.98 U/mg) was significantly reduced ($p< 0.05$) when compared with that of the normal control rats (20.17 ± 0.70 U/mg and 25.67 ± 0.76 U/mg). Activity of CAT in the kidney of treated diabetic rats with glibenclamide and *Ficus exasperata* all showed significant ($p< 0.05$) increase approaching that of the control rats when compared with the untreated diabetic rats.

Table 1:
Blood glucose concentrations of rats in experimental groups.

Groups	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Group A	78.7±5.3	81.2±5.6	78.7±4.8	81.3±5.0	79.5±5.0	78.3±2.2
Group B	424.3±16.8	454.5±27.2	472.0±27.6	495.0±20.8*	503.3±16.2*	520.3±19.2*
Group C	511.5±28.7	454±28.4*	486.8±30.1	498.2±33.8	486.8±33.8	475.8+30.4*
Group D	434.7±26.5	429.5±29.5	383.5±65.7	379.0±70.6	312.7±47.0*	274.0±47.7*
Group E	539.3±7.2	521.3±25.3	468.3±43.4	455.2±45.3	433±41.7*	400.3±31.9*

*Values are expressed as means (±SEM) of 6 rats. *Significant difference ($p<0.05$)*

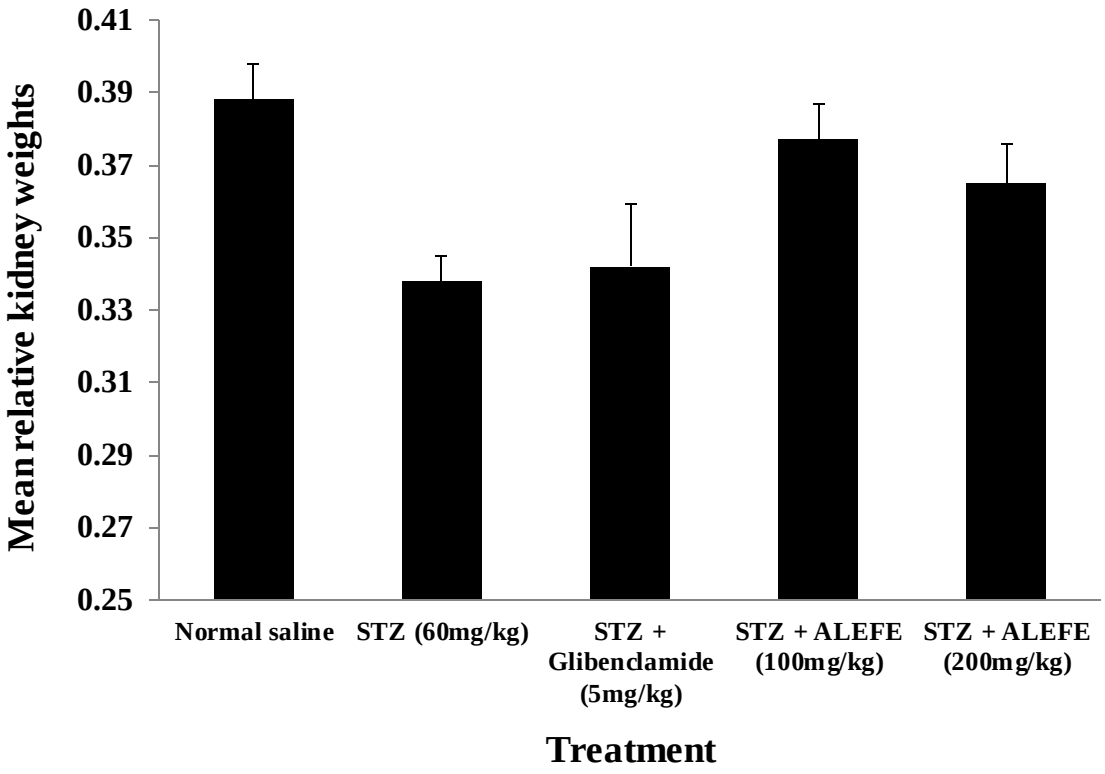

Figure 1:
Mean relative kidney weights in control and diabetic rats with or without aqueous leaf extracts of *Ficus exasperata* (ALEFE). Each bar represents Mean + SEM of 6 rats per group.

Table 2
Renal function Parameters in control and diabetic rats with or without aqueous leaf extracts of *Ficus exasperata* (ALEFE).

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	HCO_3 (mEq/l)
A	0.72±0.11	38.83±4.37	137.67±0.76	3.77±0.06	116.67±3.57	15.00±0.37
B	1.82±0.10 [a]	100.67±6.00 [a]	137.50±1.12	3.28±0.13 [a]	100.00±5.77 [a]	20.33±0.95 [a]
C	1.68±0.25 [a]	83.67±11.87 [a]	138.50±0.62	3.67±0.15 [b]	102.50±3.82 [a]	18.33±1.28
D	1.37±0.24[b]	64.00+11.74 [b]	135.00±0.37	3.35±0.11 [a]	105.67±3.82 [a]	20.00±1.26
E	1.45±0.16[a]	71.00±9.32 [b]	137.50±0.34	3.73±0.10 [b]	110.00±2.58 [a]	16.33±0.55

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

Glibenclamide failed to significantly increase the levels of SOD in Group C rats to the level of normal control rats when compared to that of the untreated diabetic rats. However, both levels of *Ficus exasperata* significantly ($p < 0.05$) increased the levels of CAT and SOD in treated diabetic rats of Groups D and E respectively when compared to the untreated diabetic rats. The concentration of Malonyldialdehyde (MDA) in the kidney of untreated diabetic rats (167.67 ± 0.92 Umol/g) was significantly high ($p < 0.05$) when compared with that of the normal control rats (73.33 ± 0.95 Umol/g). Administration of glibenclamide and *Ficus Exasperata* to diabetic rats significantly ($p < 0.05$) reduced the level of MDA close to the levels of the normal control rats when compared with the untreated diabetic rats.

Table 3:
Kidney tissue antioxidant parameters in control and diabetic rats with or without aqueous leaf extracts of *Ficus exasperata*

Antioxidants	Group A	Group B	Group C	Group D	Group E
Catalase (U/mg)	20.17±0.70	3.50±0.43 [a]	7.33±0.56 [a, b]	15.83±1.01 [b]	12.00±1.37 [a, b]
SOD (U/mg)	25.67±0.76	13.17±0.98 [a]	15.83±0.70 [a]	21.17±1.81 [b]	20.00±1.88 [b]
MDA (Umol/g)	73.33±0.95	167.67±0.92 [a]	143.33±4.19 [a, b]	89.83±1.17 [b]	122.33±3.67 [a, b]

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

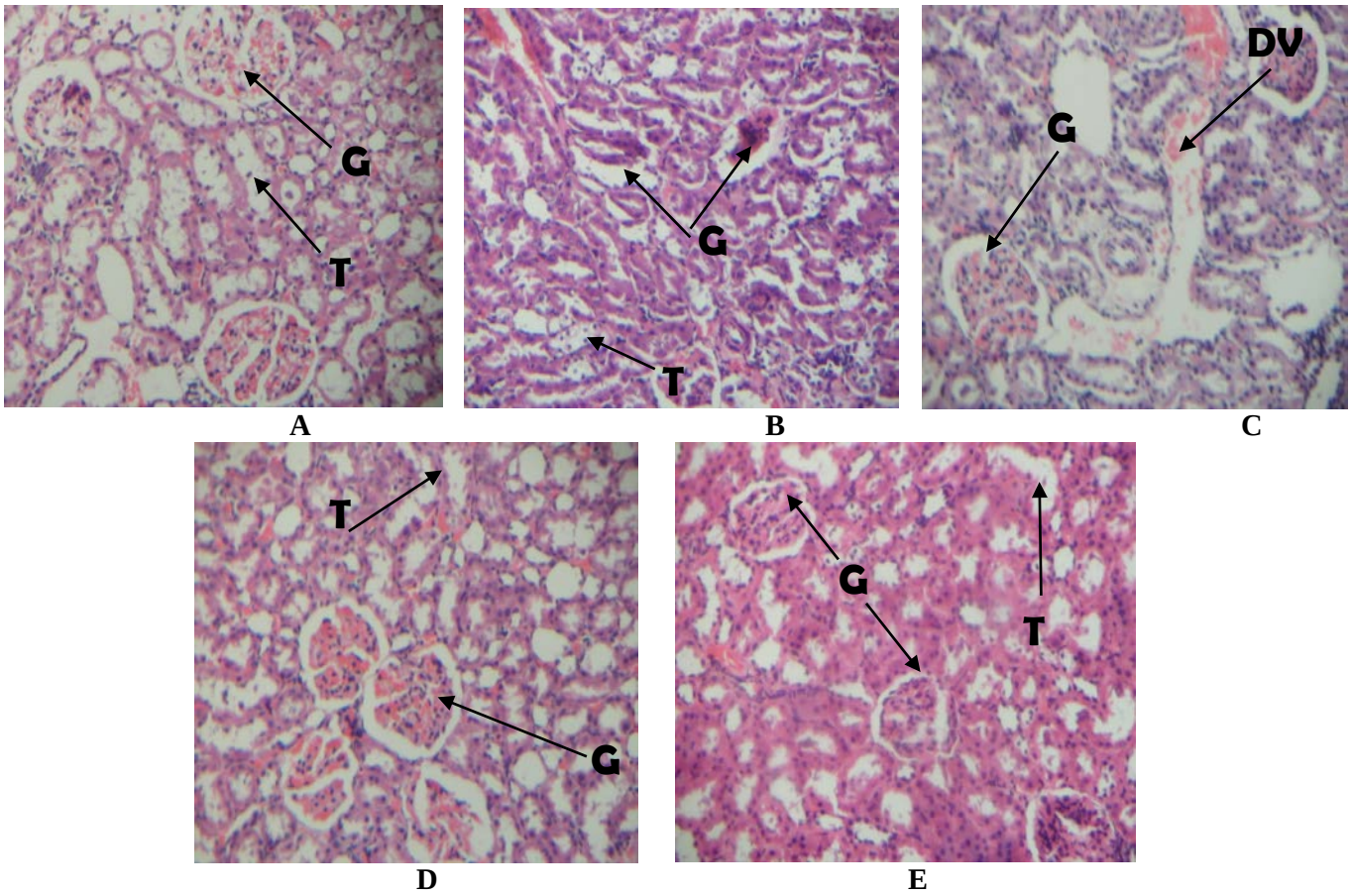

Plate 1
Photomicrographs of the kidneys of control and diabetic rats with or without aqueous leaf extracts of *Ficus exasperata* (ALEFE). A- Kidney Tissue (Group A control) showing Glomeruli (G) and Tubules (T). Also showing typical spread of glomerular tuft with even lining of glomerular space (H&E X 100). B- Kidney Tissue (Group B Diabetic group administered with 60mg/kg STZ) showing abnormal Glomerular Tufts (G) compressed into nodules with enlarged glomerular spaces, eroded Tubular cells (T) with isolated nuclei as well as collapsed tubules. C- Kidney Tissue (Group C diabetic rats treated with 5mg/kg glibenclamide) showing regular tuft of Glomeruli (G) and dilatation of vessels (DV) in the parenchyma.(H&E X 100). D- Kidney Tissue (Group D diabetic rats treated with 100mg/kg *Ficus exasperata* extract) showing apparent normal Glomeruli (G) and Tubules (T). (H&E X 100). E- Kidney Tissue (Group E diabetic rats treated with 200mg/kg *Ficus exasperata* extract) showing fairly normal Glomeruli (G) and Tubules (T) that appear normal. (H&E X 100).

Morphological Changes in the Kidney

The histological findings showed that there was visible distortion in the architecture of the kidney tissue due to the administration of Streptozotocin (STZ). The histology of the normal control group (Plate 1a) showed normal renal cortex, showing glomeruli, tubules and interstitium. This was remarkably different from that of the untreated STZ induced diabetic group (Plate 1b) which showed compression of the glomerular

tuft into nodules. These distortions were significantly reduced on administration with glibenclamide for two weeks where sections showed fairly normal glomeruli and dilatation of the vessels in the parenchyma (Plate 1c). On treatment of the diabetic groups with both doses of *Ficus exasperata*, sections began to show well patterned renal architecture and well-arranged glomeruli and tubules (Plate 1d-e).

DISCUSSION

The Kidney is an important physiological organ which when damaged may lead to alterations in metabolic activities. It is well known that increased reactive oxygen species (ROS) resulting from oxidative enzyme breakdown have been implicated in diabetes and its complications (Vats *et al.*, 2004). To overcome increased ROS, an intrinsic scavenging system, which includes antioxidants and antioxidant enzymes play a vital role. It has been reported that hyperglycemia leads to generation of ROS in tissues from glucose auto-oxidation and protein glycosylation (Ajabnoor, 1990) thereby altering normal cellular defense mechanisms and eventually leading to increased oxidative stress. Several reports have also suggested that increased free-radical mediated oxidative stress is involved in diabetic complications (McGarry, 2002).

Administration of *Ficus exasperata* leaf extract reduced the activities of Superoxide dismutase (SOD) and Catalase (CAT) in diabetic rats. The result of the SOD and CAT activities clearly shows that *Ficus exasperata* contains a free radical scavenging activity, which could have a beneficial activity against pathological alteration caused by ROS. In comparison with this study, Cho *et al.* (2002) recorded an increased SOD and CAT activity in the liver of STZ-induced diabetic rats. The effects of glibenclamide drug administration to STZ induced diabetic rats resulted in significant increase in renal SOD and CAT activities as compared to the untreated STZ induced diabetic group.

Also, treatment of the diabetic rats with aqueous *Ficus exasperta* leaf extracts produced a significant increase in both renal SOD and CAT activities. The result shows that *Ficus exasperata* extract was more effective than glibenclamide in restoring the selected biochemical variables towards normal. Malondialdehyde (MDA) is one of the lipid peroxidation products frequently used to determine the oxidant/antioxidant balance in diabetic patients (Cheeseman and Slate, 1993). The present study revealed that the kidney MDA level increased significantly after intraperitoneal injection of STZ as compared to the control group. After treatment with *Ficus exasperata* extracts, the level of renal MDA of the diabetic rats showed significant decrease, as compared to the untreated diabetic group. Furthermore, treatment with glibenclamide drug also showed significant decrease in liver MDA level of diabetic rats.

The results suggest that aqueous extracts of *Ficus Exasperata* leaves or glibenclamide drug may effectively normalize the impaired antioxidants status in STZ-induced diabetes. Additionally, *Ficus exasperata* might be a more powerful free radical scavenger than glibenclamide. It is well known that weight loss in diabetics may be due to the loss in adipose tissue and muscle which results from excessive fatty acid and tissue protein breakdown (Granner, 1996). One of the symptoms present in diabetes mellitus is weight loss which occurs when there is poor glycaemic control. Several reports have shown significant weight loss/reduction in untreated diabetic rats (Ahmed et. al., 2005; Enogieru *et. al.*, 2014). Furthermore Enogieru et. al., (2014) also reported appreciable body weight increase in diabetic rats which were treated with aqueous extracts of *Ficus exasperata*.

A decrease in relative kidney weight was observed in all animals induced with diabetes. There was no significant difference in the relative kidney weights of glibenclamide treated and *Ficus exasperata* treated diabetic rats when compared with the normal control rats. The observed effect of this extract on relative kidney weight had been earlier reported (Ijeh and Ukweni, 2007). Urea levels in diabetic control was significantly higher with respect to the normal rats. Observation of creatinine reactivity behaved similarly to that of urea. Creatinine is a metabolite of muscle creatine, whose amount in serum is proportional to the body's muscle mass. The amount of creatinine is usually constant, so that elevated levels indicate diminished renal function only, since it is easily excreted by the kidneys (Loeb, 1991). Present observations on the kidney sections showed progressive damage associated with the severity of hyperglycaemia. Severe hyperglycaemia induced by the streptozotocin caused the renal damage. Diabetic kidneys as observed in this study were prone to derangements. The changes at the molecular level impacted on the gross architecture of the kidney tissues. A comparison of the plates (histopathology) of the rat kidney revealed the degeneration of the glomerular capsule and obliteration of glomerular tuft in the untreated diabetic group. These changes were virtually were reversed following *Ficus exasperata* extract treatment. These pathologic changes were induced by the oxidative stress associated with diabetes. Jimoh and Odutaga (2004) had reported alterations and disintegration of the glomeruli of kidneys as a consequence of free radicals generated by thermo-oxidised lipids. It is likely that these pathological changes led to disruption in filtration and

concentration of urine, as well as fluid and electrolyte balance, as was observed from biochemical assays. Administration of *Ficus exasperata* extract reversed the antioxidant and biochemical changes associated with oxidative stress-hyperglycemia, hence the corrective measure observed in the histology of the kidney.

This study suggests therefore, that extracts of *Ficus exasperata* besides its ameliorative action could protect the kidneys against impairment due to diabetes. Furthermore, these findings proffer preliminary biochemical and histological support to the ethno medicinal uses of the plant in the management and/or control of diabetes mellitus

REFERENCES

Aebi H. (1984): Catalase *in vitro*. *In*: Methods in Enzymology, Academic Press, New York, pp.479-500.

Ahmed N. (2005): Advanced glycationendproducts-role in pathology of diabetic complications. *Diabetes Research & Clinical Practice*., 67, 3-21.

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

Ajabnoor M. A. (1990): Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. J Ethnopharmacol; 28, 215-20.

Cheeseman, K. H., Slate T. F. (1993): An introduction to free radical biochemistry. Br. Med. Bull. 49, 481-493.

Cho S. Y., Park J. Y., Park E. M., Choi M. S., Lee M. K., Jeon S. M., Jang M. K., Kim M. J. Park Y. B. (2002): Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. Clin. Chim. Acta, 317, 109-117.

Deniz S., Arzu S., Figen I., Gulden C. (1997): Lipid peroxidation and antioxidant status in experimental animals. Effects of aging and hypercholesterolemic diet. Clin. Chem. Acta, 265, 77-82.

Drury, R.A.B., Wallington, E.A., Cameron, R.C. (1976): Histological techniques: 4th ed., Oxford University Press NY. U.S.A. pp. 279-280.

Enogieru A. B., Omoruyi S. I., Momodu O. I., Baxter-Grillo D. (2014): Assessment Of The Effect Of *Ficus Exasperata* (Vahl) Aqueous Leaf Extracts On Body Weight And Serum Liver Enzymes In Hyperglycaemic Wistar Rats. J Med. & Biomed. Res. 13(1), 110-119

Granner D. K. (1996): Hormones of the pancreas and gastrointestinal tract; Harper's biochemistry; 25th ed. 610-626; New York: McGraw Hill.

Ijeh I. I., Ukweni A. I. (2007): Acute effect of administration of ethanol extracts of *Ficus exasperata* vahl on kidney function in albino rats. J Med. Plants Res. 1, 27-29

Jimoh F. O., Odutuga A. A. (2004): Histological changes of selected rat tissues following the ingestion of thermally oxidized groundnut oil. Biokenistri 16, 1-10

Kameswararao B., Kesavulu M. M., Apparao C. (2003): Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats. Fitoterapia 74, 7-13

Kang K.S., Kim H.Y., Yamabe N., Nagai R., Yokozawa T. (2006): Protective effect of sun Ginseng against diabetic renal damage. Biological &. Pharmaceutical Bulletin, 29, 1678-1684.

Kim H. J., Kong M. K., Kim Y. C. (2008): Beneficial effects of Phellodendri Cortex extract on hyperglycemia and diabetic nephropathy in streptozotocin-induced diabetic rats. BMB. Rep. 41(10), 710-15

Loeb S. (1991): Clinical Laboratory test: Values and Implication. Copyright, Springhouse Corporation, Springhouse, Pennsylvania.

McGarry J. D. (2002): Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes. 51, 7–18.

Montilla P., Barcos M., Munoz M., Castaneda I., Tunez I. (2005): Red wine prevents brain oxidative stress and nephropathy in streptozotocin-induced diabetic rats. J. Biochem. Mol. Biol., 38(5), 539-544.

Ogbera A. O., Adedokun A., Fasanmade O.A., Ohwovoriole A. E., Ajani M. (2005): The foot at risk in Nigerians with diabetis mellitus. The Nigerian scenario. International journal of Endocrinology and metabolism 4,165-173

Ogbonnia S. O., Odimegwu J. I., Enwuru V. N. (2008): Evaluation of hypoglycemic and hypolipidemic effects of ethanolic extracts of Treculia africana Decne and Bryophyllum pinnatum Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats. Afr. J. Biotechnol., **7**(15), 2535-2539

Petal M., Rybczynslti P. (2003): Treatment of non-insulin dependent diabetes mellitus. Expert Opin.Inveslig. Drugs. 12(4), 623-633

Sawiress (2011): Effect of Ginseng Extract Supplementation on Renal Functions in Diabetic Rats. Journal of Agricultural Science. 3(2), 17-31

Sun Y., Oberley L. W., Li Y. A. (1998): Simple method for clinical assay of superoxide dismutase. Clin. Chem. 34, 479-500.

Vats V., Yadav S. P., Grover J. K. (2004): Effect of T. foenumgraecum on glycogen content of tissues and the key enzymes of carbohydrate metabolism. J Ethnopharmacol. 90, 155-60

Wild S., Roglic G., Green A., Sicree R., King H. (2004): Global prevalence of diabetes: estimates for the year 2000 and projections for 2030". Diabetes Care 27(5), 1047-53