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A Comparative Study on the Effects of Ethanol Bark and Methanol Leaf Extracts of *Kigelia africana* on Some Biochemical Parameters in Alloxan Induced Diabetic Rats

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Abstract: A comparative study on the effect of ethanol bark and methanol leaf extract of *Kigelia africana* on some biochemical parameters in alloxan induced diabetic rats, was carried out. The ethanol bark and methanol leaf extracts of this plant was fed to alloxan-induced diabetic rats for a period of 14days and its effect on some biochemical parameters on the blood serum of the rats were assayed. Twenty-five (25) male albino rats were divided into five groups: normal rats (group 1), diabetic untreated rats (group 2), diabetic rats treated with glibenclamide (group 3), diabetic rats treated with ethanol bark extract (group 4) and diabetic rats treated with methanol leaf extract (group 5), with groups 1 and 2 serving as positive and negative control respectively. The treatment groups were orally administered 300mg/kg of each extract and their serum analysed for fasting blood sugar (FBS), MDA, Alanine aminotransferase (ALT) and Creatinine levels. The result reviewed significant (p<0.5) decreases in fasting blood sugar (FBS) level, MDA, ALT and creatinine in the rats feed with the extract when compared to group 2 (diabetic untreated group). This indicates that the extracts contain antioxidants which mop up free radicals in the system as seen in the decrease in FBS, malondialdehyde (MDA), ALT and creatinine. Going by the results of this study, the ethanol bark extract seems to be more effective than the methanol leaf extract. This supports the claim in different parts of the country that the plant is effective in the management of diabetes and its related complication.

Key words: Kigelia africana · Oxidative Stress · Diabetes · Malondialdehyde and Fasting Blood Glucose

INTRODUCTION

Our world harbors rich sources of medicinal plants which are used in the treatment of diseases [1]. Human use of these plants as medicinal agent pre-dates recorded history [2]. These medicinal agents from nature has been a source of drugs for thousands of years and an impressive number of modern drugs have been isolated from these natural sources [3]. Their presence in modern drugs is justified as they serve as extremely useful natural drugs, providing basic compounds that are less toxic, more effective and their inactive products can be modified using suitable biological and chemical means into potent drugs [4]. Plants and herbs are staging a comeback all over the globe as people are returning to the naturals with hope of safety and security, herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment [5]. Also many synthetic drugs are not only expensive and inadequate for the treatment of diseases, but also often with many adulterations and side effects [6].

Diabetes mellitus remains one of the age-long chronic diseases of the human race and its frontiers are expanding by the day. It is a metabolic diseases characterized by chronic hyperglycemia due to defective insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid and protein metabolism [7,8]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels [9]. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure and/or

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neuropathy with risk of foot ulcers, amputation and features of autonomic dysfunction, including sexual dysfunction [9].

Kigelia africana is widely used in African herbal medicine, the fruit is believed to be a cure for a wide range of ailments, from rheumatism, snakebites, syphilis and even evil spirits [10]. The aqueous leaves extract of *Kigelia africana* has been confirmed to possess anti-diarrhoeal activity [11]. The roots, bark, leaves, stems, twigs and fruits are used to treat digestive disorders and are taken as a laxative or emetic, to treat chronic and acute digestive disorders and against gastric infections [12]. The ethanol extract of the steam bark was examined to show strong analgesic and anti-inflammatory activities. The extract components inhibited the synthesis of prostaglandins and other inflammatory mediators which probably accounted for the analgesic and anti-inflammatory properties [13]. Based on these data, the present study aims to evaluate the comparative study on the effects of ethanol bark and methanol leaf extracts of *Kigelia africana* on some biochemical parameters in alloxan induced diabetic rats.

MATERIAL AND METHODS

Plant Material: The leaf and bark of *Kigelia africana* were collected from Omor, Ahamelu Local Government Area, Anambra State of Nigeria. The fruits and leaves were authenticated by the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Extraction of Plant Materials: The leaves and bark of *Kigelia africana* were air-dried at room temperature for four weeks after which it was grounded into fine powder. A quantity of 500g each of the powdered form of the leaves and bark of *Kigelia africana* were macerated in 1.5 litres of methanol and ethanol respectively for 48h. The solution was filtered with Whatman no 4 filter paper and the filtrate was concentrated to a semi solid residue in an oven of 60°C.

Experimental Animal: Adult Wistar Albino rats between 12 to 14 weeks of age, with average weight of 108 ± 4g were obtained from the Department of Veterinary Medicine and housed in the animal House of the Department of Home Science and Dietetics, both in University of Nigeria, Nsukka. The animals were acclimatized for 7 days under standard environmental conditions, with a 12 hour light / dark cycle maintained on a regular feed (Top feed; grower mash) and water.

Experimental Induction of Diabetes: The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150 mg/kg body weight and the route of administration was intraperitoneal. Blood samples were taken from the tail vein 72 h after the alloxan injection to measure the blood glucose levels by ACCU-Check glucose meter. Animals with blood glucose levels (After fasting for 12 h) over 200 mg/dl were considered diabetic and used for the further study. The treatment lasted for fourteen (14) days in which blood glucose levels of the animals were determined at the beginning and at the end of the study.

Experimental Design: Twenty five (25) all male Wister albino rats weighing 100-190g were used for the study. They were acclimatized for fourteen (14) days with free access to food and water. They were evenly distributed into five (5) groups of five (5) rats each.

Group 1: Normal control
Group 2: Diabetic untreated rats.
Group 3: Diabetic rats treated with 2.5 mg/kg body weight glibenclamide
Group 4: diabetic rats treated with 300 mg/kg body weight ethanol bark extract
Group 5: Diabetic rats treated with 300 mg/kg body weight methanol leaf extract

At the end of the experimental period the rats were starved for 12 h and then sacrificed under ether anaesthetized. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m to separate serum. Serum was carefully separated into dry clean Wassermann tubes, using a Pasteur pipette and kept frozen at (-20°C) until estimation of some biochemical parameters.

Estimation of the Chosen Biochemical Parameters: All the chosen biochemical parameters were estimated using biodiagnostic kits and the procedures were strictly followed as outlined in the manual guide.

Statistical Analysis: Data were reported as means ± SEM, where appropriate. Both one- and two- way analysis of variance (ANOVA) were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p < 0.05$.

RESULTS

Effect of the Ethanol Bark and Methanol Leaf Extracts of *Kigelia Africana* on Fasting Blood Sugar (Fbs) of Alloxan Induced Diabetic Rats: A significant (p<0.05) increase was observed in the FBS level of all the groups as depicted in the red bar indicating that the rats were all diabetic except group 1 which served as the normal control. The administration of various dosages of standard drug, ethanol bark and methanol leaf extracts significantly (p<0.05) reduced the FBS level when compared to group 2 (Diabetic untreated) as depicted in the green bar. The ethanol bark extract (Group 4) showed more promising reduction in FBS of the diabetic rats compared to the methanol leaf extract.

Effect of the Ethanol Bark and Methanol Leaf Extracts of *Kigelia Africana* on Serum Mda of Diabetic Rats: A significant (p<0.05) increase was observed in the serum MDA level of all the groups as depicted in group 2 but the administration of various dosages of standard drug, ethanol bark and methanol leaf extracts significantly (p<0.05) reduced the serum MDA level caused by diabetes in the experimental rats.

Effect of the Ethanol Bark and Methanol Leaf Extracts of *Kigelia Africana* on Serum on Serum Alanine Aminotransaminase (Alt) of Rats: A significant (p<0.05) increase was observed in the serum ALT level of all the groups as depicted in group 2 but the administration of various dosages of standard drug, ethanol bark and methanol leaf extracts significantly (p<0.05) reduced the serum ALT level caused by diabetes in the experimental rats. The ethanol bark extract (Group 4) showed more promising reduction compared to the methanol leaf extract.

The Effect of Ethanol Bark and Methanol Leaf Extracts of *Kigelia Africana* on Serum Creatinine of Rats: A significant (p<0.05) increase was observed in the serum creatinine level of all the groups as depicted in group 2 but the administration of various dosages of standard drug, ethanol bark and methanol leaf extracts significantly (p<0.05) reduced the serum creatinine level caused by diabetes in the experimental rats.

DISCUSSION

Hyperglycaemia is associated with the generation of reactive oxygen species leading to oxidative stress which causes damage particularly to the heart, kidney, eyes, nerves, liver, small and large vesicles and gastrointestinal system. The induction of diabetes using alloxan has been described as a useful experimental model for studying the effect of hypoglycemic agents [14-16]. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, resulting in the destruction of pancreatic beta-cells and diabetes [17].

The untreated diabetic rats had a significantly higher FBS level compared to the normal control. This confirms induction of diabetes by alloxan [14-16]. Administration of the graded doses of *Kigelia africana* stem and leaf extracts to the diabetic rats reduced the FBS levels to near normal. Although the extract mechanism of action is unknown, the reduction in the blood glucose levels in fasted normal and alloxan induced diabetic rats could be due to increased pancreatic insulin secretion from existing beta-cell of the pancreas [18]. The extents of changes in insulin levels could also be attributed to the phytochemical constituents of the extract and may account for the observed hypoglyceamic effect since they have been found to stimulate the secretion of insulin [19, 20]. From figure 1, the groups treated with glibenclamide (Group3), ethanol bark (Group 4) and methanol leaf (Group 5) extract of *Kigelia africana* showed a significant (p<0.05) decrease in FBS level when compared to the diabetic untreated group.

Increase in MDA values indicates the level of lipid peroxidation in the system. From figure 2, the groups treated with glibenclamide (Group3), ethanol bark (Group 4) and methanol (Group5) leaf extract of *Kigelia africana* showed a significant (p<0.05) decrease in MDA level when compared to the diabetic group 2. This implies that the plant extracts is as effective as the standard drug glibenclamide in managing the MDA level of the test animals, thereby reducing lipid peroxidation caused

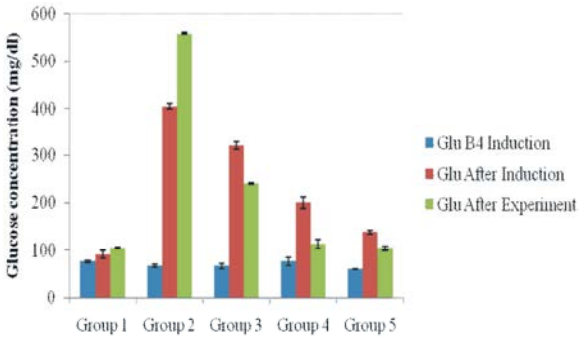

Fig 1: The fasting blood sugar level of alloxan induced diabetic rats at different stages of the experiment.

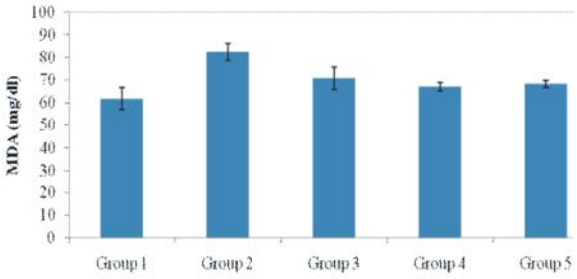

Fig 2: The effect of ethanol bark and methanol leaf extracts of *Kigelia africana* of diabetic rats.

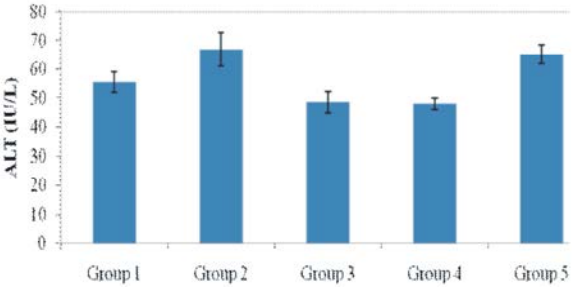

Fig 3: Effect of ethanol bark and methanol leaf extracts of *Kigelia africana* on serum alanine aminotransaminase (ALT) of diabetic rats.

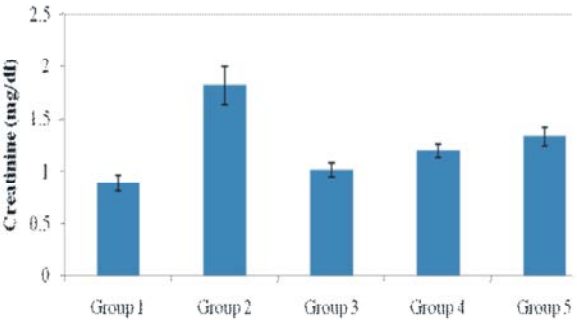

Fig 4: The effect of ethanol bark and methanol leaf extracts of *Kigelia africana* on serum creatinine of diabetic rats

by induction of diabetes. This observation is in accordance with the report of Carey *et al.* [10] that the plant played protective roles in oxidative stress conditions.

ALT is normally present in many tissues and body fluids especially in the liver. ALT is released in large amount into the serum as a result of tissue injury to hepatic cells [21] and [22]. In this study, only the groups treated with glibenclamide (Group3) and ethanol bark (group 4) extract of *Kigelia africana* showed a significant (p<0.05) decrease in ALT level when compared to the diabetic group 2 (Figure 3). This implies that the ethanol bark extract is as effective as the standard drug, glibenclamide in managing the ALT level of the test animals, thereby reducing hepatic damage caused by lipid peroxidation of hepatic cells as a result of induction of diabetes.

Creatinine is a very specific index of renal function. Elevated level of creatinine is linked with kidney damage [23]. In this study, group 4 and 5 showed significant (p<0.05) decreases in creatinine levels when compared to group 2 (diabetic untreated). This finding suggests that ethanol bark and methanol leaf extracts of *Kigelia africana* could have a protective effect on the damaged kidney brought about by alloxan induction.

CONCLUSION

The ethanol bark and methanol leaf extracts of *Kigelia africana*, were as effective as the standard drug glibenclamide in managing the negative effects of alloxan induced diabetes. Also, the ethanol bark extract seems to more efficacious than the methanol leaf extract. Therefore, the plant extracts shows a promising alternative in the management of diabetic related complications.

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