

Effect of Methanol Leaf and Fruit Extracts of *Kigelia Africana* on Some Biochemical Parameters of Normal Albino Rats

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Abstract: This study was aimed at evaluate the effect of methanol leaf and fruit extracts of *Kigelia africana* on normal rats. The catalase, vitamin C, protein level, total cholesterol, High Density Lipoprotein, Low Density Lipoprotein, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Malondialdehyde (MDA) concentration were determined. The experimental design comprises of twenty rats divided into four groups of five rats each. Group I served as a control while group II received 2.5mg/kg of glibenclamide, group III received 500mg/kg methanol leaf extract of *Kigelia africana* and group IV received 100mg/kg methanol fruit extract of *Kigelia africana*. The result showed a significant ($p < 0.05$) difference in protein level and vitamin C concentration in groups III and IV when compared with group I and group II. For total cholesterol, ALT and MDA concentration, a significant ($p < 0.05$) decrease in the treated groups (II, III and IV) was observed when compared to the normal (group I). Significant ($p < 0.05$) increase was observed in LDL concentration in group II when compared to group I, III and IV. There was no significant ($p < 0.05$) difference in HDL, AST and catalase for the different groups. The extracts showed significant reduction in, AST and lipid peroxidation (MDA), serum cholesterol as well as low density lipoprotein. The results obtained, suggest that methanol leaf and fruit extracts of *K. africana* could be meaningfully employed in maintaining and stabilizing normal metabolic state in normal organisms.

Key words: *Kigelia africana* • Lipid peroxidation • Normal rats • Lipid profile and Liver enzymes

INTRODUCTION

The body system is very sensitive to changes, no matter how minute the change may be. Under favorable conditions, there is an adequate and proper body metabolism; the body is thus said to be in normal condition. However, under any change (shift), especially in the blood glucose level, the body is exposed to either hyperglycemia (high blood sugar) or hypoglycemia (low blood sugar). In such condition, the normal body metabolism is impaired; hence, the body becomes vulnerable to diseases. Susceptibility to a group of metabolic diseases is common when one has high blood sugar (hyperglycemia), caused either because the pancreas does not produce enough insulin, or the cells do not respond to the insulin produced [1].

Free radicals are generated as by-product of normal cellular metabolism. However, several conditions disturb the balance between reactive oxygen species and cellular defense mechanism [2]. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The level of antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes.

Kigelia africana plant has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include iridoids, flavonoids, naphthoquinones and volatile constituents etc [3]. The major iridoids found in the root-bark and stem-bark of this plant is specioside, verminoside and minecoside [4]. These iridoids specifically verminoside, have had the

most scientific literature published on their unique anti-inflammatory properties. In vitro assays showed that verminoside had significant anti-inflammatory effects and inhibits both NOS (Nitric Oxide Synthase) expression and NO (Nitric Oxide) release in microphage cell lines [5].

The flavonoid luteocin has been isolated from the fruits and the leaves [6]. Literature survey indicates that there is no scientific evidence to support the normoglycemic study of the effect of the plant. Therefore, the present study was undertaken to study the possible normoglycemic effect of the methanol extracts of the leaf and fruit on normal rats.

MATERIALS AND METHODS

Plant Material: The leaf and fruit 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.

Experimental Animal: Adult Wistar Albino rats between 12 to 14 weeks with average weight of $108 \pm 4g$ 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.

Extraction of Plant Materials: The leaves and fruits of *Kigelia africana* were air-dried at room temperature for four weeks after which it was grounded into fine powder using grinding machine, 300g of the grounded leaves and fruits were separately macerated with 500ml of methanol for 48hrs. The extracts were filtered and the filtrate was used for the experiment.

Experimental Design: A total of twenty rats were used. Treatment was administered daily orally at a dose of 500mg/kg for 14 days. The animals were divided into four groups of five rats in each group as follows:

Group 1: Normal control

Group 2: Normal rats fed with 2.5mg/kg glibenclamide.

Group 3: Normal rats fed with 500mg/kg of *Kigelia africana* methanol leaf extract.

Group 4: Normal rats fed with 500mg/kg of *Kigelia africana* methanol fruit 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.

Statistical Analysis: Data were reported as means \pm 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

The Effect of Methanol Leaf and Fruit Extract of *Kigelia africana* on Catalase Activity: The result reveals that there was no significant difference ($P > 0.05$) on the catalase activity after treatment, on comparing the treated groups with group I and II.

The Effect of Methanol Leaf and Fruit Extract of *Kigelia africana* on Vitamin C Concentration in Rats: The result revealed that there was significant increase ($p < 0.05$) on vitamin C concentration after treatment, on comparing group 3 and group 4 when compared with group I and II.

The Effect of Methanol Leaf and Fruit Extract of *Kigelia Africana* on Protein Level in Rats: The result revealed that there was significant increase ($P < 0.05$) on protein level in group 3 and group 4 when compared with group I and II

The Effect of Methanol Leaf and Fruit Extracts of *Kigelia africana* on Total Cholesterol in Rats: The result revealed a significant decrease ($P < 0.05$) on the total cholesterol after treatment, on comparing group 2 (treated with glibenclamide), group 3 (treated with leaf extract) and group 4 (treated with fruit extract) with group 1 (normal control).

The Effect of Methanol Leaf and Fruit Extract of *Kigelia africana* on Hdl Concentration in Rats: The result reveals that there was no significant difference ($P>0.05$) on the HDL concentration in group IV after treatment when compared to the controls (groups I and II). Group III (treated with leaf extract) showed a significant decrease ($p<0.05$) when compared to the controls and group IV (treated with fruit extract).

The Effect of Methanol Leaf and Fruit Extract of *Kigelia Africana* on LDL Concentration in Rats: The result reveals that there was significant decrease ($P<0.05$) on the LDL concentration after treatment, on comparing the treated groups; group 2 (treated with glibenclamide), group 3 (treated with leaf extract) and group 4 (treated with fruit extract) to group 1 (normal control). That of group IV was much pronounced when compared to group III.

The Effect of Methanol Leaf and Fruit Extract of *Kigelia Africana* on AST Concentration in the Different Rats: The result reveals that there was no significant difference ($P>0.05$) on the AST concentration after treatment, on comparing the treated groups; group 2 (treated with glibenclamide), group 3 (treated with leaf extract) & group 4 (treated with fruit extract), with group 1 (control). The same is true when the extracts; group 3 (treated with leaf extract) and group 4 (treated with fruit extract), were compared with the standard drug; group 2 (treated with glibenclamide).

The Effect of Methanol Leaf and Fruit Extract of *Kigelia africana* on ALT Concentration in the Different Rats: The result reveals that there was significant decrease ($P<0.05$) on the ALT concentration after treatment, on comparing group 3 (treated with leaf extract) and group 4 (treated with fruit extract) with group 1 (normal control) and group 2 (treated with glibenclamide).

The Effect of Methanol Leaf and Fruit Extract of *Kigelia Africana* on MDA Concentration in the Different Rats: The result reveals that there was significant decrease ($P<0.05$) on the MDA concentration after treatment, on comparing group 3 (treated with leaf extract) and group 4 (treated with fruit extract) with group 2 (treated with glibenclamide). The same is true when group 3 (treated with leaf extract) and group 4 (treated with fruit extract) was compared with group 1 (normal control).

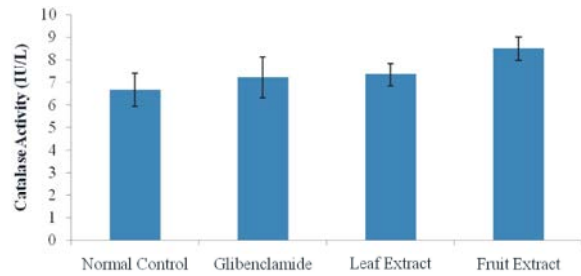


Fig. 1: Effect of methanol extracts of *K. africana* on Catalase activity in normal rats.

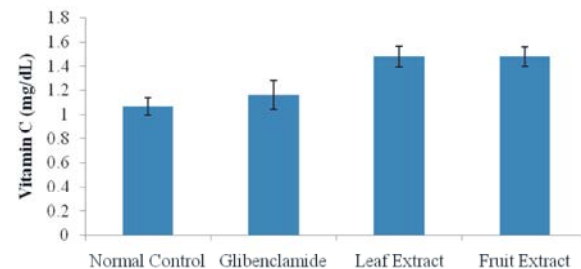


Fig. 2: Effect of methanol extracts of *K. africana* on Vitamin C concentration in normal rats.

DISCUSSION

From the results obtained for catalase activity ($P>0.05$), it becomes evident that, like the normal (group 1) and the standard drug (group 2), decreasing catalase activity is uncommon with extract of *K. africana* (group 3 and 4). Free radical scavenging enzymes like catalase protects the biological system from oxidative stress [7]. Vitamin C is essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen oxidative stress; a substrate for ascorbate peroxidase in plants (APX is plant specific enzyme) and an enzyme cofactor for the biosynthesis of many important biochemicals. Vitamin C acts as an electron donor for important enzymes [8]. The result ($P<0.05$) showing higher vitamin C concentration of group 3 (leaf extract) and group 4 (fruit extract) when compared to group 1 (normal) and group 2 (glibenclamide) is indicative of the plants richness in vitamin C. According to [9], *Kigelia africana* contain 3mg of vitamin C per 100g consumed.

The result also showed a significant ($p<0.05$) increase in the protein concentration (Figure 3) at both test doses compared to the normal control group. This suggests that the extract can be used to reduce the glucose concentrations by the formation of glycoproteins which involves the covalent attachment of carbohydrate groups to many different

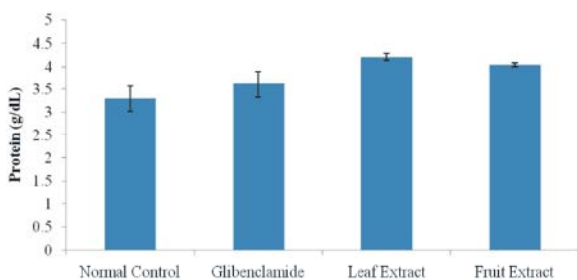


Fig. 3: Effect of methanol extracts of *K. africana* on Protein level in normal rats

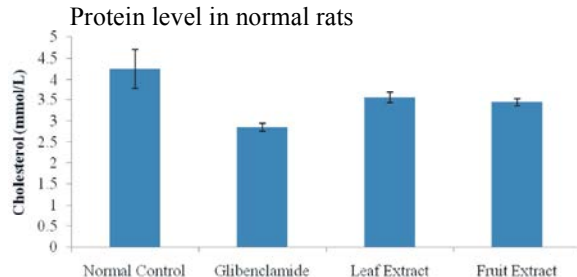


Fig. 4: The Effect of methanol extracts of *K. africana* total cholesterol in normal rats

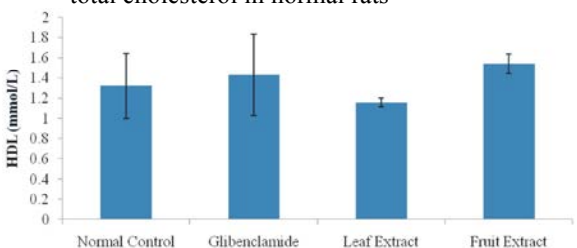


Fig. 5: Effect of methanol extracts of *K. africana* on HDL concentration in normal rats

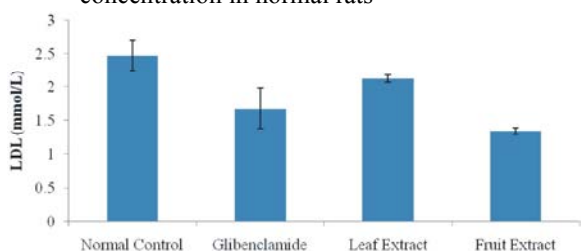


Fig. 6: Effect of methanol extracts of *K. africana* on LDL concentration on the normal rats

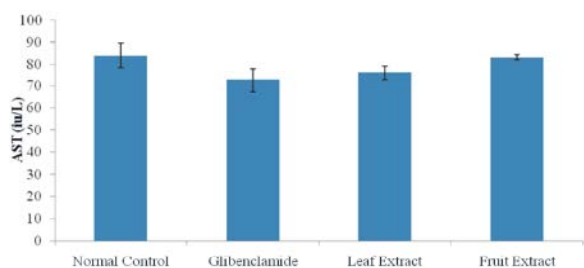


Fig. 7: The Effect of methanol extracts of *K. africana* on AST concentration in normal rats

proteins. The glycoproteins are components of cell membranes where they play a variety of roles in processes such as cell adhesion [10].

Lipids play a vital role in the pathogenesis of oxidative stress. The increased level of serum lipid in oxidative stress represents a risk factor for coronary heart disease [11]. In disease state, the concentration of serum free fatty acids is elevated as a result of free fatty acids out flows from fat depots where the balance of the free fatty acid esterification triacylglycerol lipolysis cycle is displaced in favour of lipolysis [12]. Thus an excess fatty acid in the plasma promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances along with excess TGs formed in the liver may be discharged in the liver in the form of lipoproteins [13]. HDL is an anti-atherogenic lipoprotein; it transports cholesterol from peripheral tissues into the liver and thereby acts as a protective factor against coronary heart disease. Oral administration of the methanol extracts of *Kigellia africana* leaf and fruit lower serum lipids and also increased the serum HDL-cholesterol level in normal rats as shown in Figures 4, 5 and 6. These results suggest that the extracts might be considered as a substitute of drugs to combat oxidative state associated complications.

The liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid homeostasis and is severely affected in disease state [14]. In this study, there was mark elevation in the liver marker enzymes in experimental rats which caused a marked elevation in the levels of serum AST and ALT which is indicative of hepatocellular damage. This might possibly be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage. Several studies have reported similar elevation in the activities of serum AST and ALT in disease states [15]. Oral administration of the methanol extracts of *Kigellia africana* leaf and fruit significantly reduced the activities of the above marker enzymes in normal rats as shown in Figs 7 and 8. This indicates that extracts may tend to prevent liver damage by maintaining the integrity of the plasma membrane thereby suppressing the leakage of the enzymes through the membrane, exhibiting hepatoprotective activity. A number of scientific reports indicate that certain flavonoids, terpenoids and steroids have protective effect on liver due to its antioxidant

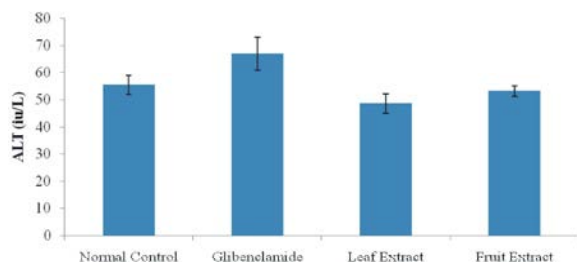


Fig. 8: Effect of aqueous extract of *K. africana* on ALT concentration in the normal rats

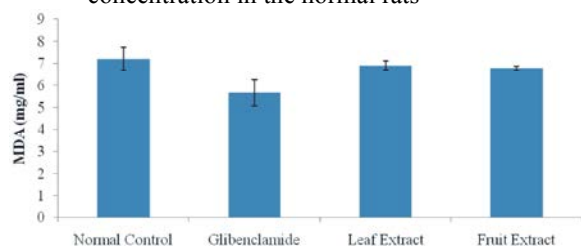


Fig. 9: Effect of aqueous extract of *K. africana* on MDA concentration in the different rats

properties [16]. Phytochemically, the extracts contain these antioxidants and may have played active role in hepatoprotective activity.

The increased lipid peroxidation in apparently healthy rats as found in the present study may be due to the inefficient antioxidant system prevalent in normal physiological conditions. The status of lipid peroxidation as well as altered levels of certain endogenous radical scavenger is taken as direct evidence for oxidative stress [17]. Free radical scavenging enzymes like SOD and catalase protects the biological system from oxidative stress [7] and [9]. The decrease in the activity of the enzymes in the present study could be attributed to the excessive utilization of these enzymes in attenuating the free radicals generated during normal physiological conditions. Similar reports have shown an elevation in the status of lipid peroxidation in the liver disease state [9] and [10] and our results are in accordance with these reports. Therefore, the antioxidant properties of the extracts may have resulted in the recoument in the activities of the enzymatic antioxidants (catalase). Non-enzymatic antioxidants acts synergistically to scavenge the free radicals formed in the biological system.

CONCLUSION

Methanol leaf and fruit extract of *K. africana* extract have shown significant reduction in free radical related complications, lipid peroxidation (MDA),

blood cholesterol as well as low density lipoprotein. This suggest that the extract could extracts be meaningfully employed in maintaining and stabilizing normal metabolic state in normal organisms. Further studies should be carried out on prolonged treatment of the plant extract is then suggested. This is to ascertain its prolonged effect on the liver and kidneys of rats. Also, more human and animal experimental studies are needed to clarify the toxicity of the methanol leaf and fruit of *Kigellia africana*.

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