Effects of Ethanol Leaf and Fruit Extracts of *Kigelia africana* on Some Oxidative and Biochemical Parameters of Alloxan -Induced Diabetic Rats.

Uhuo, E. N, Ezeanyika, L.U.S and Ogugua, V.N

[1]Turor and Head Department Chemical Sciences, Godfrey Okoye University, Enugu, [2] Professor of Biochemistry, University of Nigeria Nsukka, [3] Senior Lecturer, Department of Biochemistry University of Nigeria Nsukka

ABSTRACT

Hyperglycaemia, a characteristic feature of diabetics mellitus leads to decreased antioxidant defense and hence the development of oxidative stress, which is involved in the aetiology of development of diabetic complications. This work was therefore aimed at evaluating the anti diabetic and antioxidative potential of the plant. These evidences suggest that good glycemic control and/or use of antioxidants may play an important role in the prevention of complications associated with diabetes. Diabetes was induced with single Intra peritoneal injection of alloxan (160 mg/kg b.w) dissolved in freshly prepared citrate buffer (pH 4.5). Oral administration of Kingelia africana (500 mg/kg b.wt) of methanol leaves and fruits extracts resulted in significant (p>0.05) decrease in the blood glucose level, MDA, glycosylated haemoglobin, lipid profiles and liver maker enzymes with corresponding increase in SOD activity, catalase activity, glutathione activity, serum protein concentration, and Vit.C concentration. In conclusion, K. africana possessed antioxidative properties evidenced by decrease blood glucose level and its effect on some oxidative parameters of diabetic rats.

Keywords: Diabetes, Alloxan, Antioxidant, Kingelia africana.

INTRODUCTION

Globally, the estimated incidence of diabetes and projection for year 2030, as given by International Diabetes Federation is 350million (Ananda *et al*., 2012). Currently available pharmacotherapies for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these drugs do not restore normal glucose homeostasis and they are not free from side effects (Bandaware *et al*., 2011). In view of the adverse effects associated with the synthetic drugs and as plants are safer, affordable and effective, conventional antidiabetic plants can be explored (Kumar *et al.,* 2010). Over 400 traditional plants have been reported for the treatment of diabetes (Ramachandra *et al.,* 2011). Furthermore, following World Health Organization recommendations, investigation of hypoglycemic agents from medicinal plants has become more important (Kumar *et al*., 2010). Also, diabetes has been treated orally with several medicinal plants based on folklore medicine since ancient times.

Kigelia africana (Lam.) Benth (Family: Bignoniaceae) plant has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include iridiods, flavonoids, naphthoquinones, volatile constituent, etc. (Houghton, 2002; Gormann, 2004; Asekun *et al.,* 2000).

Experimentally, the plant has shown antibacterial, antifungal, antineoplastic, analgesic, anti-inflammatory, antioxidant properties (Saini *et al.,* 2009). Crude extracts of herbs and spices and other materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Flavonoids are groups of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995).

An enhanced oxidative stress has been observed in diabetic patients as indicated by increased free radical production, lipid peroxidation and diminished antioxidant status (Baynes, 1991). In diabetes mellitus, alterations in the endogenous free radical scavenging defence mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. Oxidative stress is currently suggested as the mechanism underlying diabetes and diabetic complications. Oxidative stress may cause oxidative damage of cellular membranes and changes in the structural and functional integrity of sub-cellular organelles and may produce effects that result in the various complications in diabetic disease (Mercuri *et al.,* 2000; West, 2000; Cam *et al*., 2003; Yavuz *et al.,* 2003). Recently, there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical-induced tissue injury. Although several synthetic antioxidants are commercially available, they are quite unsafe and their toxicity is a problem of concern.

A survey of literature revealed that there is no experimental evidence of the antidiabetic effects of *Kigelia africana.* Therefore, the present work explores this and will, in addition, study its potential effect on some oxidative and biochemical parameters of alloxan-induced diabetic rats.

MATERIALS AND METHODS

PLANT MATERIALS: The leave and fruit of *Kingelia africana* were collected from Omor, Aghamelu Local Government Area, Anambra State, Nigeria. The plant was authenticated by the Department of Plant Science and Biotechnology, University of Nigeria Nsukka.

CHEMICALS: These were analytical grade products and include: ethanol methanol (BDH), ethylene diamine tetraacetate (EDTA), hydrochloric acid (BDH), sulphuric acid (BDH), Trichloloroacetic acid (TCA), 2-thiobarbituric acid (TBA), Alloxan Monohydrate (sigma-Aldrich, USA), 1-Chloro-2, 4 dinitrothiobenzene, glutathione peroxidase kit (Randox Laboratories Limited, UK), Protein kit (Randox Company, USA), Superoxide Dismutase kit (Randox Company, USA), Glycosylated haemoglobin kit (Randox company,USA), Sorbitol kit, Lipid profile kit, Glucose test (Life Scan Inc, California, USA).

EXTRACTION OF THE PLANT MATERIAL: The leaves and fruits of *K. africana* were air-dried at room temperature for after which it was grounded into powders using Rancilio Rocky (Rigtig Kaffe A/S, Skanderborg, Denmark). A quantity of 500 mg each of the powdered leaves and fruits of *K. fricana* macerated in 1.5 litres of Ethanol for 72h. The solution was filtered with Whatman no 4 and concentred using rotary evaporator (Model Modulyo 4K, England).

ANIMALS: Male Wistar Albino rats between 12 to 14 weeks of age, with an average weight of 108±5g, were obtained from the Department of Zoology, University of Nigeria Nsukka. They were housed in the animal facilities of Department of Home Science and Dietetics, University of Nigeria, Nsukka for one week before starting the experiment. The animals were allowed free excess to a standard diet, water and maintained under optimum conditions of temperature, relative humidity and light period. (12h light/12h dark).

INDUCTION OF DIABETICS: The rats were fasted (12h) prior to injection of alloxan dissolved in cold citrate buffer (pH 4.5) in a dose of 160 mg/kg intra-peritoneally. The base line blood glucose level was determined before the induction. On the fourth-day blood samples were taken from the tail vein to measure the blood glucose level using Accu-check glucose meter (Roche, Germany). Rat with blood glucose level of 200 mg/dl and above were considered diabetic and used for the study (Ogugua *et al*., 2013).

The treatment was for a period of 4 weeks in which the bloods obtained were used for parameters analysis.

EXPERIMENT BEGIN: Thirty (30) male Wister albino rats with an average weight of 108±5 g were classified into 6 groups (5 rats per group) and subjected to treatment as follows.

Group i: Normal control rats.

Group ii : Diabetics untreated rats.

Group iii: Diabetic rats treated 2.5 mg/kg bwt glibenclamide

Group iv: Diabetics rats treated with 500 mg/kg btw ethanol leaf extracts.

Group v: Diabetic rats treated with 500 mg/kg btw ethanol fruit extracted.

Group vi: Diabetic rats treated with an equal ratio of ethanol leaf and fruit extracts.

At the end of the experiment, rats were starved for 12h and blood glucose levels were determined. Blood samples were received into clean dry centrifuge tubes and use for the analysis of the parameters.

ESTIMATION OF BIOCHEMICAL PARAMETERS: All the chosen biochemical and oxidative parameters were estimated using bio-diagnostic kits and the procedures were strictly followed as outlined in the manual guide.

STATISTICAL ANALYSIS: Results were reported as mean± SEM, where appropriate. Both one-and two-way analysis of variance (ANOVA) was used to analyse the experimental data and

Ducan multiple test rage was used to compare the group means obtained after each treatment with control measurement. Significant value was taken at $p < 0.05$.

RESULTS

Qualitative Phytochemical Composition of Ethanol Leaf and Fruit Extracts of *K. africana*

Table1 Shows relative trace presence of saponin and terpenoids in all the extract samples. In the same vein, hydrogen cyanide and steroid were found to be present in trace concentrations. Relative moderate amount of soluble carbohydrates was found in all the extracts. Interestingly, flavonoid was found in high concentration in the extracts.

Table 1: Qualitative phytochemical composition of ethanol leaf and fruit extracts of *K. africana.*

Extract	Soluble carbohydrate	Tannin	Alkaloid	Hydrogen cyanide	Saponin	Flavonoid	Reducing sugar	Steroid	Glycoside	Tepenoid
Ethanol leaf	+	+	+	+	+	++	+	+	++	+
Ethanol fruit	+	++	++	+	+	+++	+	+	++	+

NB
+ Present in trace concentration
++ Present in moderately high concentration
+++ Present in very high concentration

Quantitative Phytochemical Composition of Ethanol Leaf and Fruit Extracts of *K. africana*

Table 2 shows the quantitative composition of bioactive compounds present at various concentrations. A significant increase of flavonoid was recorded in ethanol fruit compared with the leaf extracts. Trace concentration of hydrogen cyanides was found in the extracts. All the extracts contained moderate concentration of alkaloid.

Table 2: Quantitative phytochemical composition leaf and fruit extracts of *K. afrcana*.

Extract	Soluble carbohydrate (mg/100 g)	Tannin (mg/100 g)	Alkaloid mg/100 g	Hydrogen cyanide mg/100 g	Saponin mg/100 g	Flavonoid mg/100 g	Reducing sugar (mg/100 g)	Steroid (mg/100 g)	Glycoside (mg/100 g)
Ethanol leaf	0.96±0.02	4.11±0.65	2.67±0.12	0.03±0.001	0.52±0.02	2.32±0.04	50.85±3.36	0.53±0.03	2.39±0.15
Ethanol fruit	0.96±0.17	9.08±0.14	3.54±0.11	0.95±0.02	0.56±0.03	3.63±0.02	26.95±5.14	0.57±0.01	2.874±0.14

Effects of Ethanol Extracts of Leaves and Fruits of *K. africana* on Sugar Level of Diabetic Rats

The sugar levels of rats before the experiment in all groups were determined. Significant ($p<0.05$) increased sugar level of induced rats was observed compared with the sugar level the rats before the experiment as shown in **Table 3.** Reversibly, sugar level of rats after 21 days treatment reduced significantly ($p<0.05$) compared the level of sugar at 72 hours after induction and before induction.

Table 3: Effect of ethanol extracts of leaves and fruits of *K. africana* on sugar level of diabetic rats

Treatment Groups	Sugar Level (mg/dl)		
	Before Induction	**72 Hours After Induction**	**After 21 Days Treatment**
Group 1 (Normal Control)	76.20 ± 5.02^{ab}	78.80 ± 2.71^{ab}	75.40 ± 4.22^{ab}
Group 2 (Diabetic Untreated)	67.40 ± 3.50^{ab}	$558.40\pm14.01^{ac*}$	$405.40\pm15.96^{ac*}$
Group 3 (Standard Control)	66.40 ± 3.91^{ab}	$321.00\pm115.16^{ab*}$	$241.20\pm116.79^{ab*}$
Group 4 (Diabetic + Ethanol Leaf Extract)	72.20 ± 4.96^{ab}	$314.80\pm159.19^{ab*}$	184.40 ± 54.50^{ab}
Group 5 (Diabetic + Ethanol Fruit Extract)	76.40 ± 9.07^{ab}	$464.80\pm159.32^{ac*}$	$273.20\pm93.59^{ab*}$
Group 6 (Diabetic + Ethanol Leaf and Fruit Extract)	64.60 ± 3.20^{ab}	$479.60\pm142.28^{ac*}$	$269.60\pm108.64^{ab*}$

Results are expressed in mean ± SD; n = 5

Mean values with different letters as superscripts across the column compared with group 2 (diabetic untreated) are considered significant ($p<0.05$) while mean values with asterisk (*) as superscripts across the row compared with the sugar level before the experiment are considered significant ($p>0.05$)

Body Weight of Diabetic Rats Treated with Ethanol Extracts of Leaves and Fruits of *K. africana* before and after Experiment

Significant ($p<0.05$) increased in the body weight of test groups compared with diabetic rats after treatment was observed. Conversely, non-significant ($p>0.05$) decrease was observed in the body weights of the animals in other groups 6 after the experiment compared with the body weights of the animals before the experiment of the control group. (Table 4)

Table 4: Body weights of diabetic rats treated with ethanol extracts of leaves and fruits of *K .africana* before and after experiment

Treatment Groups	Body Weight (g)	
	Before Experiment	**After Experiment**
Group 1 (Normal Control)	92.59±5.87[a]	130.36±17.83[b]
Group 2 (Diabetic Untreated)	173.66±12.24[a]	156.16±13.14[a]
Group 3 (Standard Control)	94.58±5.80[a]	107.34±18.41[a]
Group 4 (Diabetic + Ethanol Leaf Extract)	103.94±8.30[a]	125.98±14.59[b]
Group 5 (Diabetic + Ethanol Fruit Extract)	119.83±40.91[a]	127.94±37.44[a]
Group 6 (Diabetic + Ethanol Leaf and Fruit Extract)	86.71±9.77[a]	95.12±4.09[a]

Results are expressed in mean ± SD; n = 5

Mean values with different letters as superscripts across the row are considered significant ($p<0.05$)

Effects of Ethanol Leaf and Fruit Extracts of *K. africana* on Sorbitol Concentration in Alloxan-Induced Diabetic Rats

Sorbitol concentration in all the test groups decreased significantly ($p<0.05$) compared with the untreated diabetic animals (Group 2). A significant ($p<0.05$) reduction of sorbitol concentration was recorded in groups 6 treated with a combination of the leaf and fruit extracts of *K.africana* compared with the diabetic untreated rats (Fig. 1).

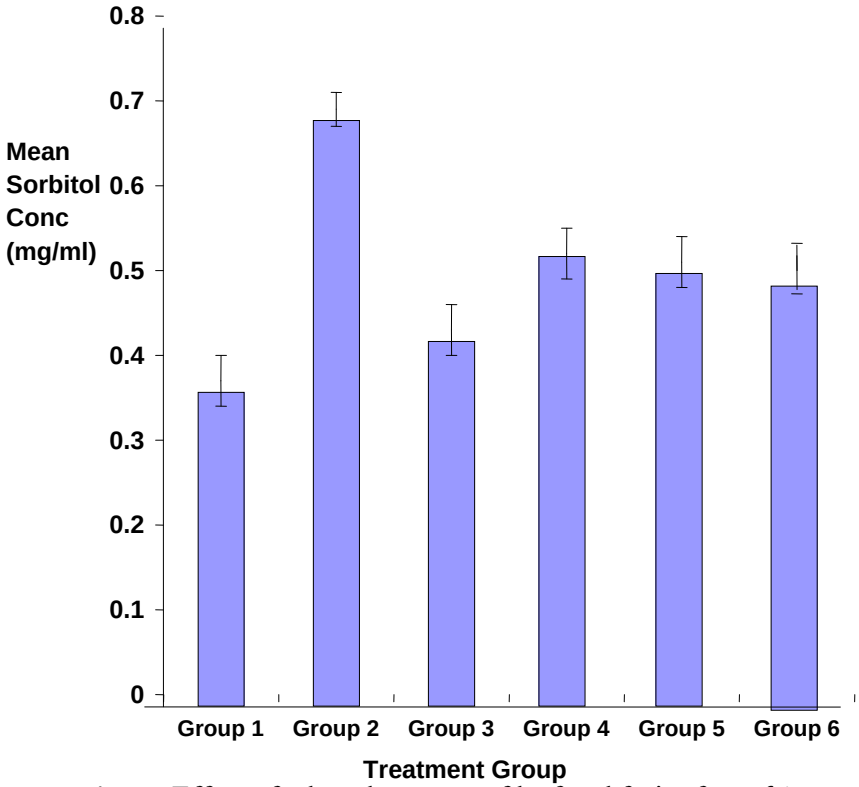


Fig. 1 Effect of ethanol extracts of leaf and fruit of *K. africana* on sorbitol concentration in alloxan-induced diabetic rats

Effect of Ethanol Leaf and Fruit Extract of *K. africana* on Total Protein Concentration in Alloxan-Induced Diabetic Rats

Fig. 2 reveals observable significant increased ($p>0.05$) of total protein in all test groups compared with the positive control rats (group 2). Total protein concentrations in groups 6 fed with a combination of the leaf and fruit extract showed significant increase ($p<0.05$) compared with other test groups.

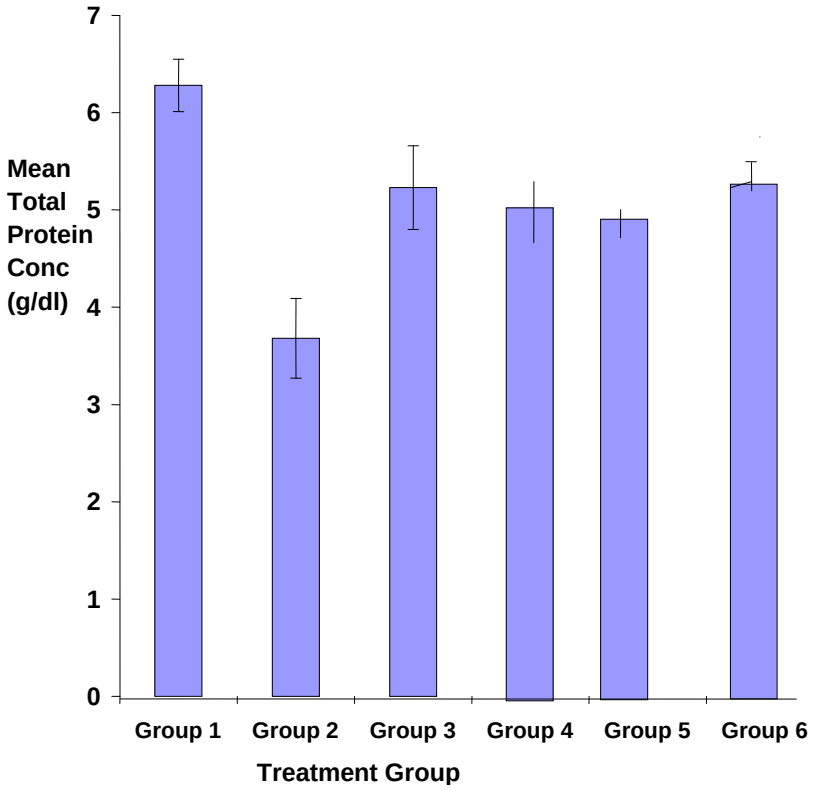


Fig. 2 Effect of ethanol extracts of leaf and fruit of *K. africana* on total protein concentration in alloxan-induced diabetic rats

Effect of Ethanol Leaf and Fruit Extract of *K. africana* on Glycosylated Haemoglobin Concentration in Alloxin-Induced Diabetic Rats

The mean HbA1c level decreased significantly ($p<0.05$) in all the test groups compared with the HbA1c level of untreated diabetics rats (group 2).Change in HbA1c level was observed in group 6 rats treated with a combination of leaf and fruit extract in ratio of 1:1 compared with group 2 rats untreated. A significant increase ($p<0.05$) HbA1c level was recorded in all the test groups against the normal control rats (Fig.3). Group 6 (diabetic+ ethanol leaf and fruit extracts, ratio 1:1) demonstrated a non-significant ($p>0.05$) reduction of HA1c concentration compared with groups 4 and 5 rats orally fed with single plant extract (**Fig. 3**).

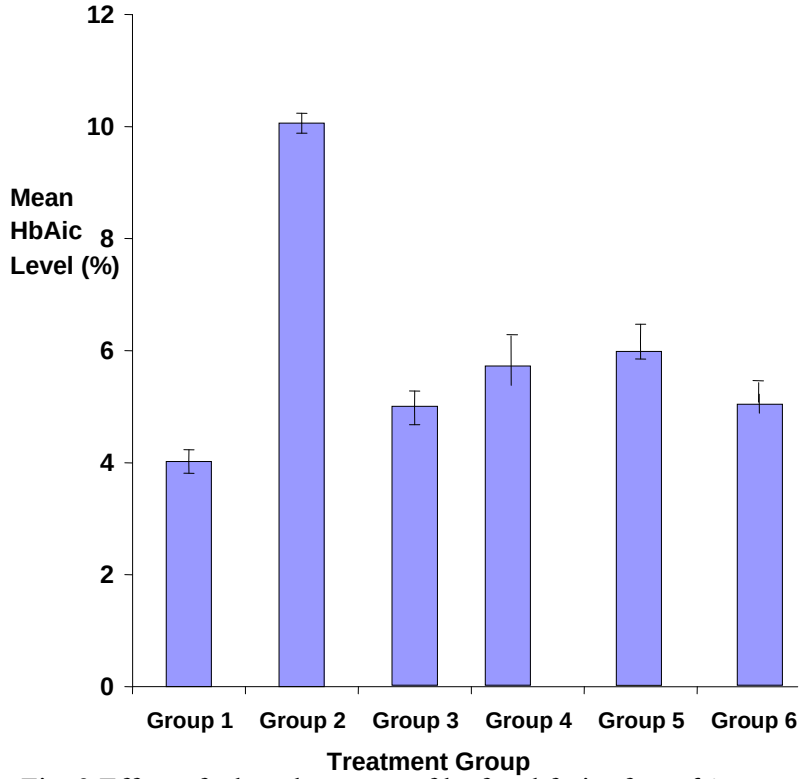


Fig. 3 Effect of ethanol extracts of leaf and fruit of *K. africana* on glycosylated haemoglobin concentration in alloxan-induced diabetic rats

Effects of Ethanol Extracts of Leaf and Fruit of *K. africana* on Malondialdehyde (MDA) Concentration in Alloxan-Induced Diabetic Rats

Lipid peroxidation measured as malondialdehyde (MDA) observed significantly increase ($p<0.5$) in all the test groups compared with untreated control as shown in **Fig.4**. A significant decrease of MDA concentration was recorded in group 6 treated with the combination of the plant extract compared with the groups administered with single extract (groups 4 and 5). Similarly, a significant decrease ($p<0.05$) of MDA concentration was observed in the test groups compared with MDA concentration of untreated diabetic group.

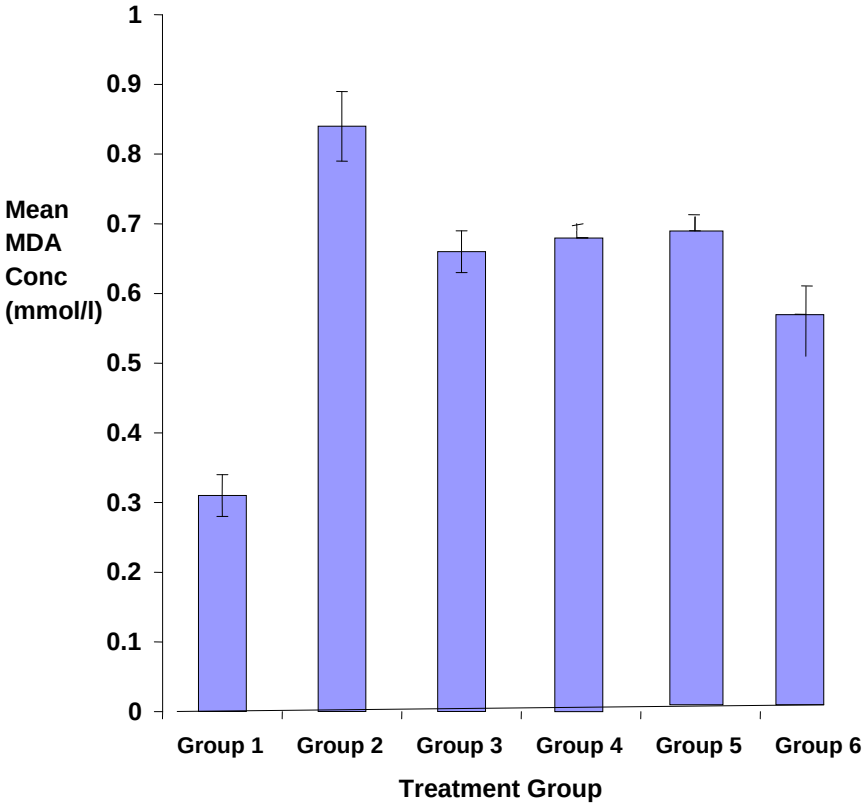


Fig. 4: Effects of ethanol extracts of leaf and fruit of *K. africana* on malondialdehyde concentration in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extracts of *K. africana* on Vitamin C Concentration in Alloxan-Induced Diabetic Rats

There was a general decrease in vitamin C concentration in all the test groups and the untreated diabetic group compared with the vitamin concentration of normal control rats (group 1). There was statistically significant increase ($p<0.05$) of vitamin C concentration in group 6 rats treated with a combination of ethanol leaf and fruit extracts compared with other test groups. The diabetic rats administered 2.5 mg/kg b.wt of glibenclamide demonstrated an increased ($p<0.05$) vitamin c level compared with the vitamin C concentration of rats in group 2 (diabetic untreated rates), see Fig.5

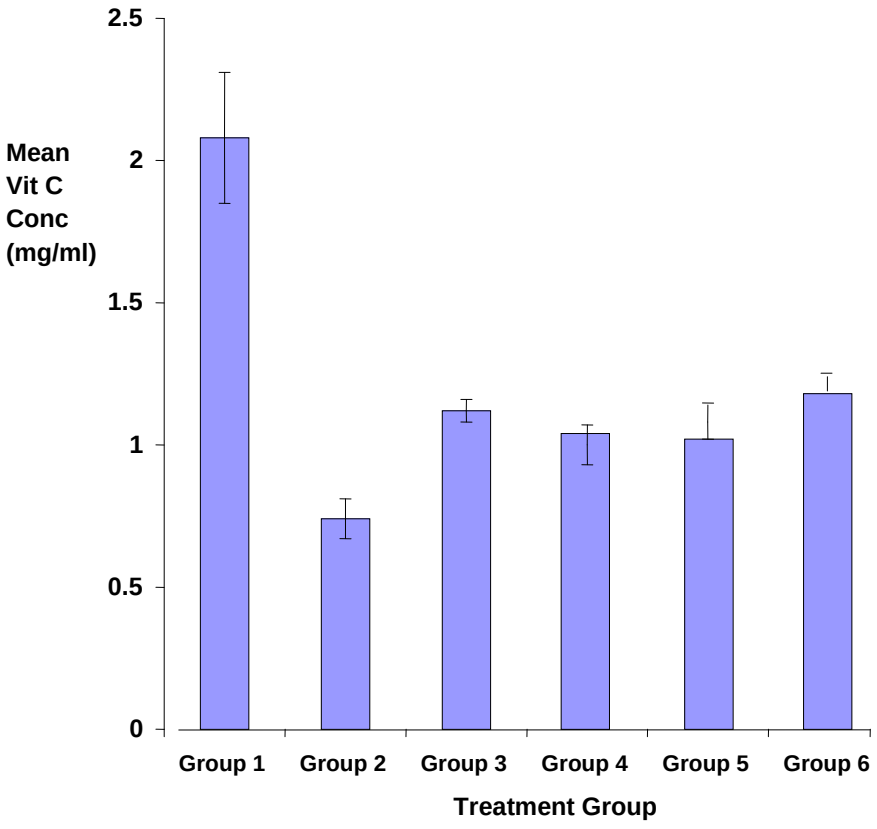


Fig. 5: Effects of ethanol extracts of leaf and fruit of *K. africana* on vitamin C concentration in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extracts of *K. africana* on Catalase Activity in Alloxan-Induced Diabetic Rats

Across the test groups was recorded a statistically significant increase ($p<0.05$) catalase activity (Fig.6) compared with the untreated diabetic rats (positive control; group 2). Similarly, a significant increase ($p<0.05$) of catalase activity was observed in the diabetic rats treated with reference drug (glibenclamide) in comparison with the catalase activity of all the test groups. In the same pattern, group 6, ethanol leaf + fruit extracts demonstrated a non significant increase ($p>0.05$) of catalase activity compared with other test groups (groups 4 and 5) administered with a single plant extract.

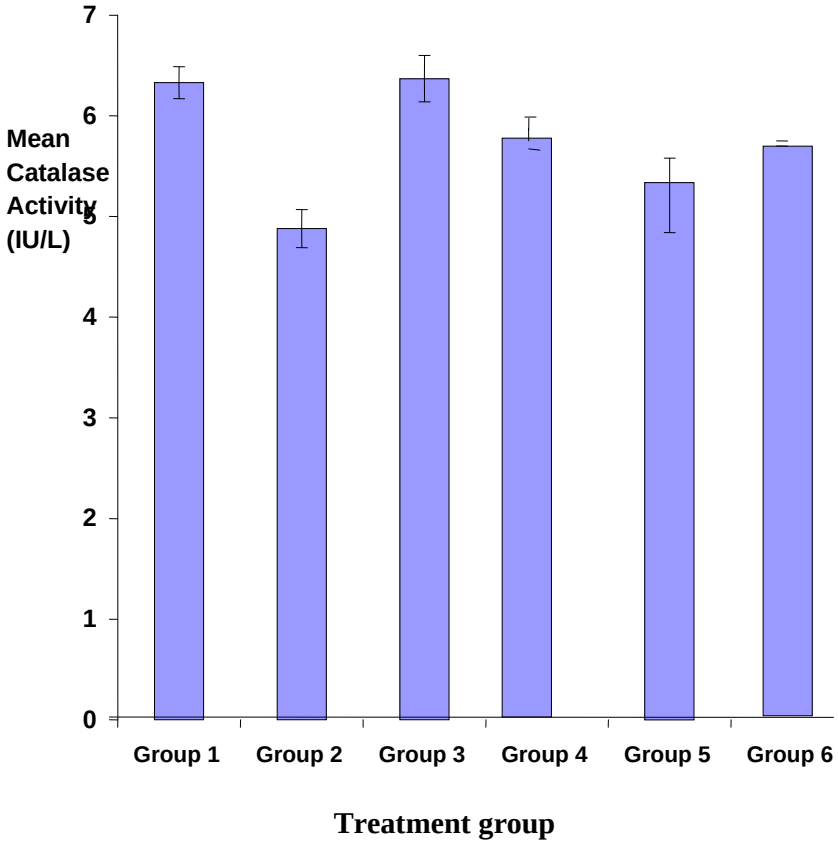
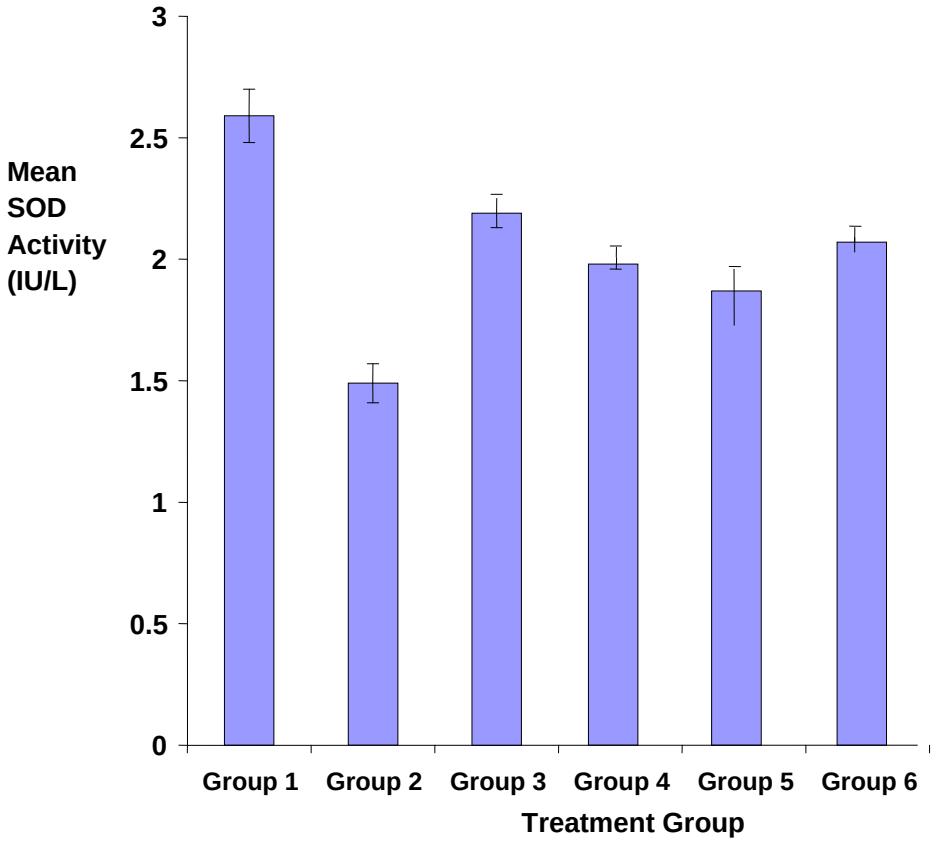


Fig. 6: Effects of ethanol extracts of leaf and fruit of *K. africana* on catalase activity in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extracts of *K. africana* on Superoxide Dismutase (SOD) Activity in Alloxan-Induced Diabetic Rats

The activities of superoxide dismutase (SOD) reduced significantly ($p<0.05$) in all the test groups compared with the normal control (group 1).There were statistically significant ($p<0.05$) increase in SOD activities of all test groups compared with the untreated diabetic rats (group 2) as shown in **Fig. 7**. Superoxide dismutase activities of the test groups (group 6) administered with the combination of the extracts was significantly increased ($p<0.05$) compared with other test groups treated with the single extracts (groups 4 and 5). The same observation was noted in the test treated with the standard drug. However, the activities of SOD in the diabetic rat administered with 2.5 mg /kg b.wt of glibenclamide increased significantly ($p<0.05$) as against all the test groups.



Fi.g. 7: Effects of ethanol extracts of leaf and fruit of *K. africana* on superoxide dismutase in alloxan – induced diabetic rats

Effects of Ethanol Leaf and Fruit Extracts of *K. africana* on Percentage Inhibition of SOD Activity in Alloxan-Induced Diabetic Rats

Fig.8 demonstrates statistically significant decrease ($p<0.05$) of percentage inhibition of SOD activity in the test groups compared with the normal control group. A significant reduction ($p<0.05$) of percentage inhibition of SOD activity occurred in the diabetic untreated rats (group 2) compared with the percentage inhibition of SOD activity in normal control. Diabetic 6 treated with a combination of leaf and fruit extracts recorded a significantly ($p>0.05$) increase of percentage inhibition of SOD activity compared with groups 4 and 5 administered monotherapically with leaf and fruit extracts of *K. africana*. Conversely, group 6 treated with ethanol leaf extracts showed significant increase ($p<0.05$) of percentage inhibition of SOD activity as against groups 4, 7 and 8 of the same treatment pattern. Furthermore, non-significant reduction ($p>0.05$) of percentage inhibition was observed in diabetic administered the n-hexane leaf and fruit extracts compared with the diabetic rats treated with 2.5 mg/kg body weight of glibenclamide (group 3).

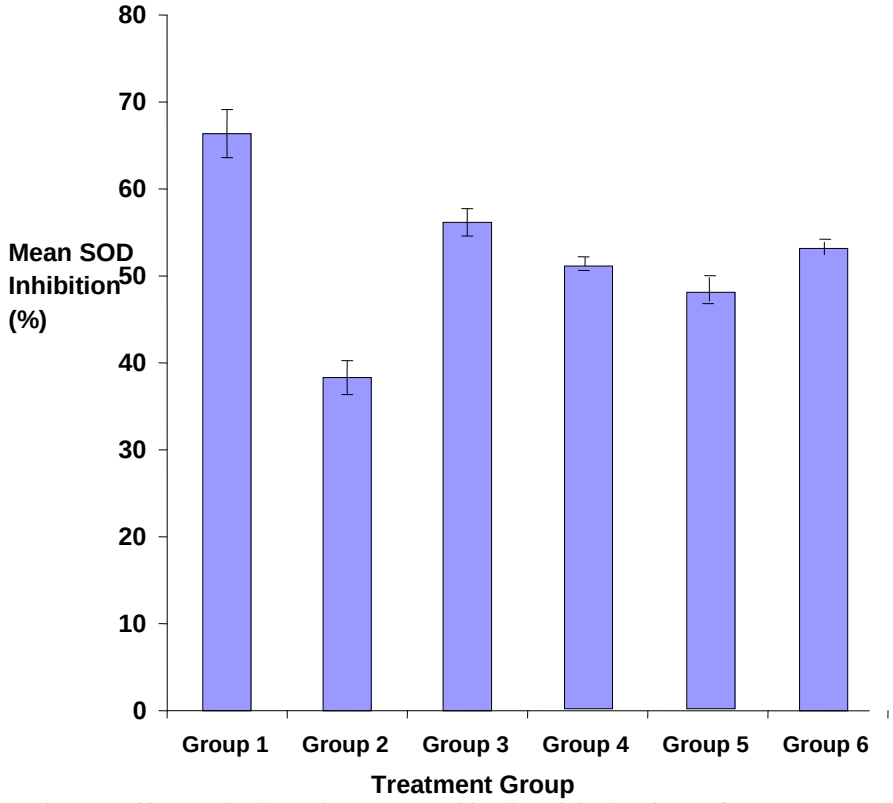


Fig. 8: Effects of ethanol extracts of leaf and fruit of *K. africana* on superoxide dismutase percentage inhibition in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extract of *Kigelia africana* on Glutathione Peroxidase Activity in Alloxan-Induced Diabetic Rats

Fig. 9 represents activity of glutathione peroxidase (GPx) which increased significantly ($p<0.05$) in all the test groups treated with both single and combination of the leaf and fruit of *K. africana* extract in comparison with the GPx activity of the rats in group 1 (normal control rats). The combination therapy in group 6 demonstrated significant increase ($p<0.05$) of GPx activity compared with groups 4 and 5 test groups treated with a single plant extract (monotherapy).In the same vein test groups 6 treated with combined leaf and fruit extracts increased in GPx activity significantly ($p<0.05$) relative to group 3 treated with the standard drug.

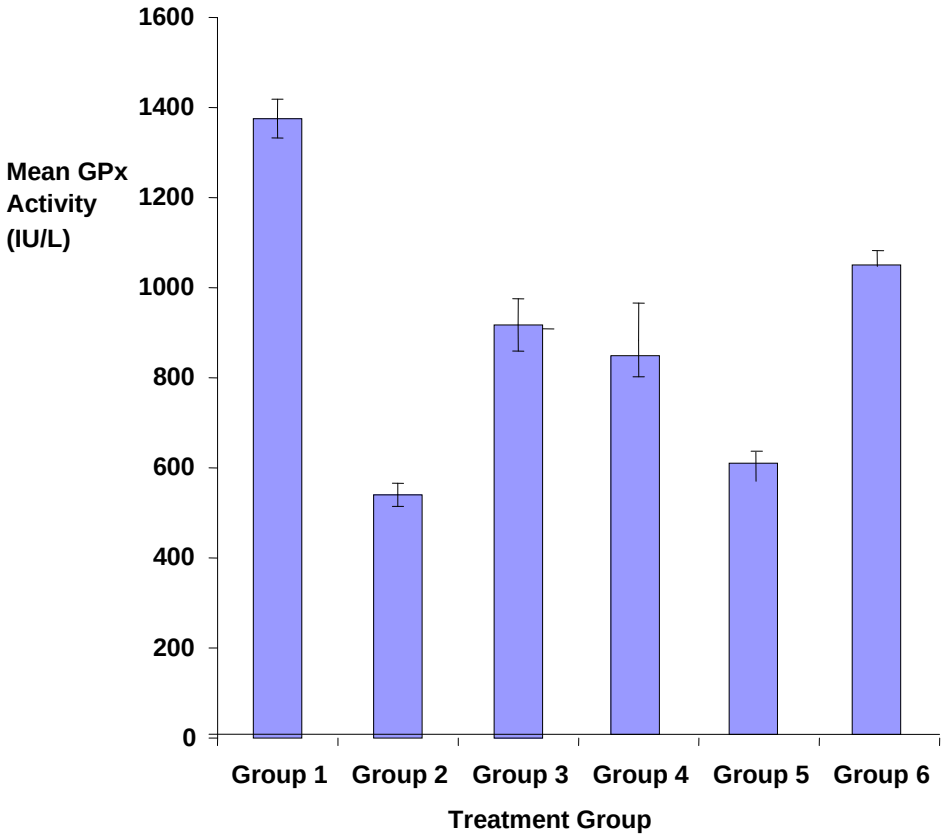


Fig 9: Effects of ethanol extracts of leaf and fruit of *K. africana* on glutathione peroxidise activities in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extract of *K. africana* on Total Cholesterol Concentration in Alloxan-Induced Diabetic Rats

Fig.10 shows relative increase in the total cholesterol concentration in diabetic test groups 4, 5 and 6 compared with the total cholesterol concentration of normal control in group 1, the increase was found to be significant ($p < 0.05$). A significant ($p<0.05$) decrease was noted in the diabetic rats administered with the standard drug compared with the untreated diabetic rats (group 2). Similar trend of result was observed in total cholesterol concentration of group 6 treated with a combination of the extracts compared with the total cholesterol concentration in diabetic untreated

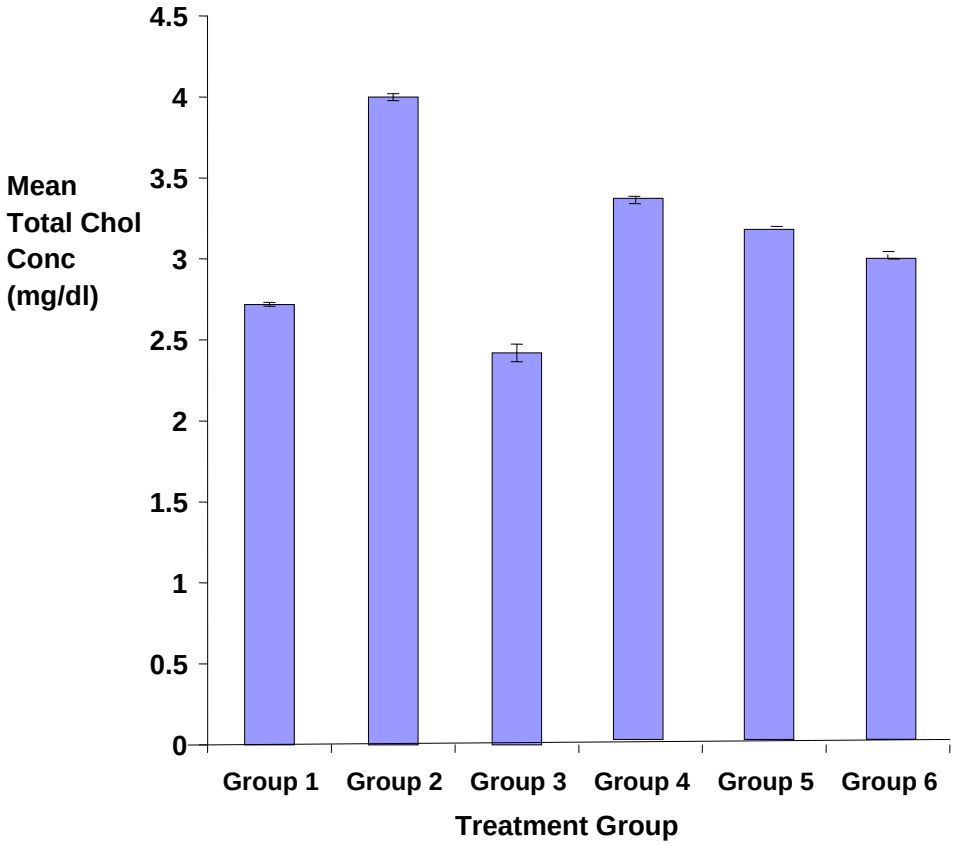


Fig. 10: Effects of ethanol extracts of leaf and fruit of *K. africana* on total cholesterol concentration in alloxan-induced diabetic rats.

Effects of Ethanol Leaf and Fruit Extract of *K. africana* High Density Lipoprotein Concentration in Alloxan-Induced Diabetic Rats

A significant reduction ($p<0.05$) of high-density lipoprotein (HDL) cholesterol was noted in groups 4 and5 treated with different single extracts of leaf and fruit of *K. africana* as shown in Fig. 11 compared with the HDL concentration of normal control rats (group 1). Conversely, the HDL concentration of rats in test groups 6 administered with a combination of ethanol leaf and fruit of equal ratio increased, though not significant ($p>0.05$) compared with the HDL concentration of rats in group 3 treated with the standard drug (2.5 mg/kg body weight). Similarly, a significant decrease ($p<0.05$) of HDL concentration was obtained in group 6 treated with a combination of the two parts of the plant compared with the diabetic untreated rats (**Fig. 11**).

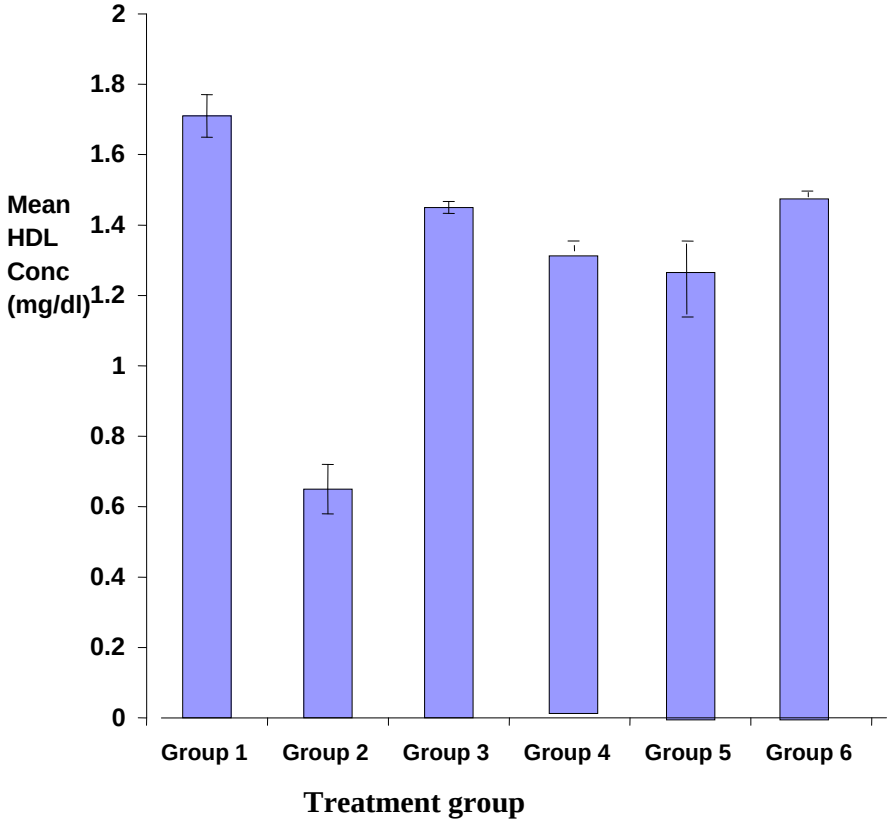


Fig. 11: Effects of ethanol extracts of leaf and fruit of *K. africana* on high density lipoprotein concentration in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extracts of *africana* on Low Density Lipoprotein Concentration in Alloxan-Induced Diabetic Rats.

Fig. 12 shows significant increase ($p<0.05$) in the concentration of low-density lipoprotein (LDL) in test groups 2 & 4 compared with the concentration of low-density lipoprotein of the control rats (group 1). Non-significant ($p>0.05$) variation of low-density lipoprotein (LDL) concentration across the test groups 4 & 5 (diabetic +single plant extract) compared the LDL concentration of rats in groups 6 treated with a combination of *K. africana* leaf and fruit extracts. Invariably, significant ($p<0.05$) decrease of LDL concentration was observed in all, except group 4 as against diabetic rats untreated (Group 2).

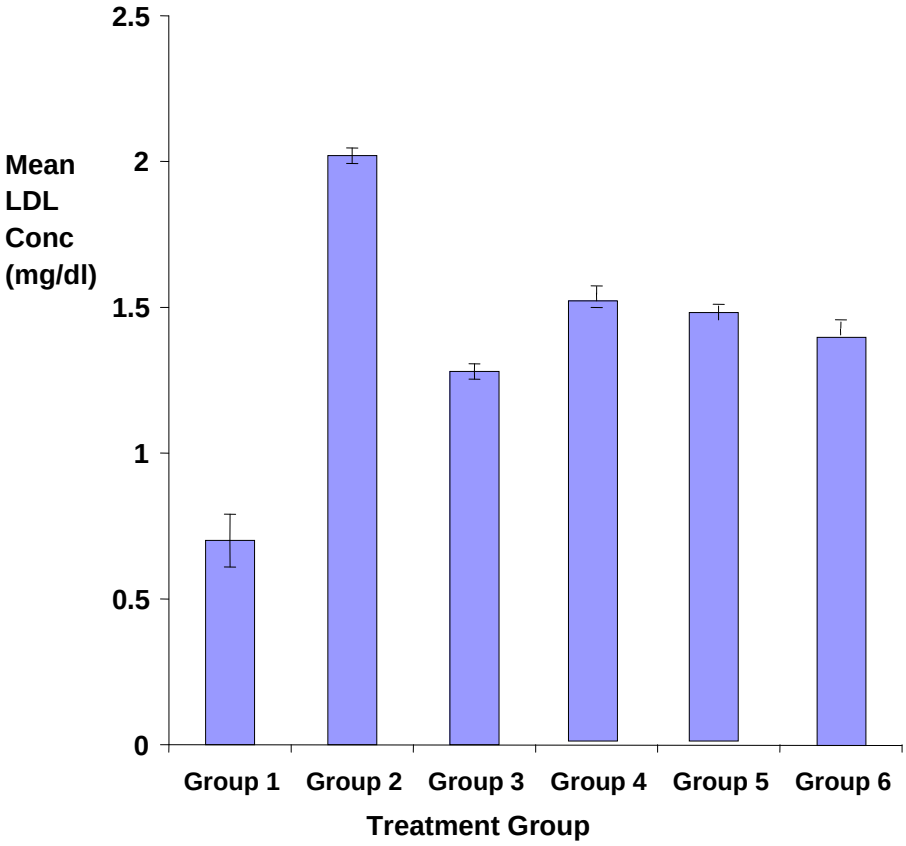


Fig. 12 Effects of ethanol extracts of leaf and fruit of *K. africana* on low density lipoprotein concentration in alloxan-induced diabetic rats

Effects of Ethanol Leaf and Fruit Extracts of *K. africana* on Triacylglycerol (TAG) Concentration in Alloxan-Induced Diabetic Rats

Triacylglycerol (TAG) concentration decreased significantly ($p<0.05$) in groups 4 to group 6) orally fed *K. africana* extracts compared with the TAG concentration of rats (group 2). Furthermore, a significant increase ($P<0.05$) of TAG concentration in group 4 and 5 was observed in comparison with normal control rats (group 1). However, a non-significant ($p>0.05$) decrease of TAG concentration in group 6 administered with a combination of leaf and fruit extracts of *K. africana* was noticed compared with groups 4 and 5 treated with single extract of the plant. Also as shown in **Fig. 13,** group 3 (standard treatment) demonstrated observable significant ($p>0.05$) changes in comparison with other test groups (4, 5 and 6).

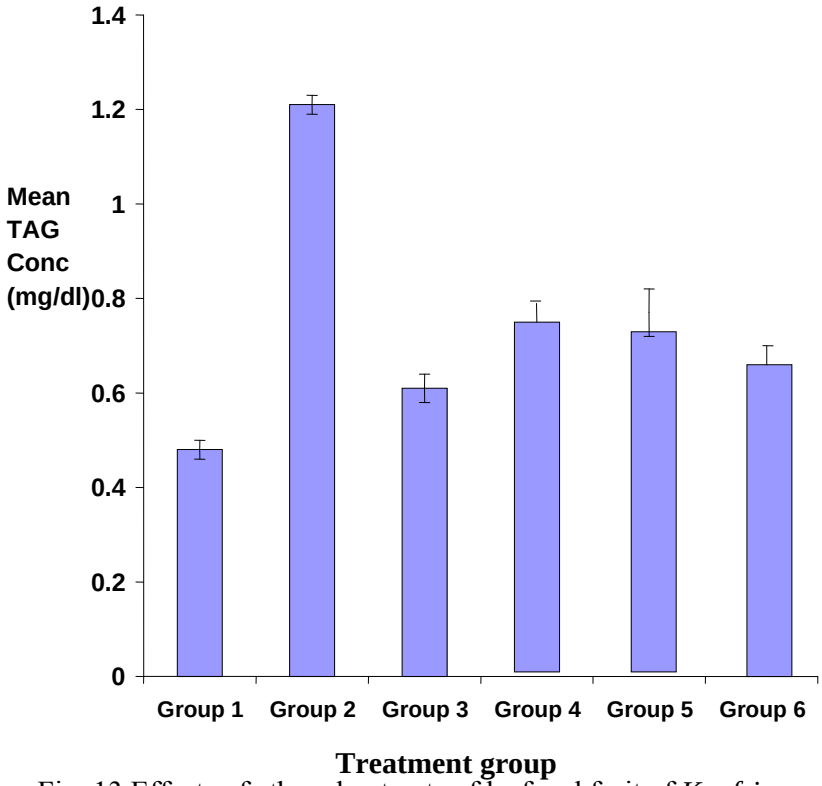


Fig. 13 Effects of ethanol extracts of leaf and fruit of *K. africana* on triacylglycerol concentration in alloxan-induced diabetic rats

Discussion

This study evaluated the antidiabetic and antioxidative properties of *Kigelia africana* in alloxan-induced diabetic rats. From the results obtained; untreated diabetic rats had much higher blood glucose level than that of the normal control. Changes in blood glucose levels reflect abnormalities in ß- cells structure and function. Alloxan causes glucose oxidation and reduction in insulin release by the destructions of ß- cells of the islets of Langerhans (Siyem *et al.,* 2002). Administration of *K. africana* ethanol leaf and fruit extracts restored glucose level in alloxan-induced diabetic rats near the normal level. Glibenclamide was used as a standard drug to compare the activity of *K. africana* extract in reference to blood glucose reduction. The results revealed that the extracts in a dose of 500 mg/kg body weight significantly ($P<0.05$) decreased blood glucose level at 21st day indicating that the extracts possessed extra pancreatic hypoglycemic activities. The comparable effect of the extract (500 mg/kg) with glibenclamide (2.5 mg/kg) may suggest a similar mode of action since the main mechanism of the action of glibenclamide is the stimulation of insulin release and the inhibition of glucagon secretion. It has been described that glibenclamide is effective in moderate diabetic state and ineffective in severe diabetic animals where pancreatic ß- cells are totally destroyed (Suba *et al*., 2004).) The possible mechanism by which the plant extract brings about its hypoglycemic action may be by potentiating the insulin effect thereby increase pancreatic secretion of insulin from ß- cells (Stanley *et al.,* 2000). The findings also suggest that *K. africana* leaf and fruit many generate ß-cells and have protective effect on ß- cells from glucose toxicity.

In general, there is little biological knowledge on the specific modes of action of plants in the treatment of diabetics, but most of the plants have been found to contain substances like glycosides, alkaloids, terpernoids, and flavonoid that are frequently implicated as having antidiabetic effect (Low and Kazkin, 2002). This was also buttressed by the results of the phytochemistry of *K. africana* which revealed high percentage of flavonoid, glycoside, alkaloid, terpernoid that are frequently implicated as having an antidiabetic effect. These plant constituents can lower blood glucose level.

Sorbitol concentration significantly decreased ($P<0.05$) across all the test animals in reference to diabetic untreated rats. This reduction is probably due to the reduction of sorbitol reductase by the extracts. Sorbitol is a product of polyol pathway and is a feature of diabetic complications. It could be suggested that some of the active constituents of *K. africana* extracts inhibit the activity of aldose reductase; the major enzyme in the polyol pathway.

This study further revealed significant reduction ($P<0.05$) of sorbitol concentration in groups 6 rats treated with the combination of leaf and fruit extracts relative to animals treated with the

reference drug (2.5 mg of glibenclamde). This is in line with the fact that synthetic drugs do not restore normal glucose homeostasis and are not free from side effect (Bandawane *et al.,* 2011).

A significant ($p<0.05$) increase in glycosylated haemoglobin level in the diabetic rats untreated with reference to the normal control animals (group 1) was recorded in this study. The increase was in accordance with the report of several other researchers (Testamarian and Cohen, 1992; Langerstroer and Pieper *et al.,* 1993; Ting *et al.,* 1996). The increased glycosylation may be as a result of diabetic complications caused by oxidative stress. Generally, decreased in glycol-haemoglobin level was observed in diabetic rats treated with *K. africana* extracts as against diabetic rats not treated. The decrease in glycol-haemoglobin level could be attributed to the extracts' ability to reduce glucose level in the blood stream.

High concentration of MDA in diabetic untreated established oxidative stress status in the animals. In hyperglycemic condition, glucose is one of the major sources of free radicals. Lipid peroxidation measured as malondialdehyde (MDA) significantly ($p<0.05$) decreased in all the test groups compared with diabetic rats untreated (group 2). Groups 6 treated with a combination of leaf and fruit extracts showed significant ($p<0.05$) decrease in MDA concentration as against groups 4-5 treated with single extract. Reduction in the lipid peroxidation index in treatment groups indicates the ability of the extracts to stem down the oxidative stress by mopping up free radical that lead to lipid breakdown. The bioactive constituents of the extracts such as flavonoids, alkaloids as revealed by photochemistry results could be implicated in free radical scavenging properties of the extracts.

A significantly increase ($p<0.05$) in serum total protein was recorded in all the test groups treated with the plant extracts in comparison with the diabetic untreated rats. The decrease in serum total protein was observed in untreated diabetic rats with reference to test groups both single and combination of the plant extracts. This is in tandem with the proximate composition of the plant that revealed approximate 13% protein.

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shirwaikar *et al*., 2006) and these contribute to coronary artery diseases. This lends credence to the significant ($P<0.05$) increase of total cholesterol, triacylglycerol and low-density lipoprotein in the diabetic rats used in this study. *K. africana* treated rats showed a reduction in these lipids which buttressed the hypolipidemic effects of the plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Kumar *et al*., 2011). It could also be attributed to the increase in the reverse cholesterol transport pathway and decreased cholesterol concentration in the intestine due to α- glycosidase inhibition. Administration of a combination of leaf and fruit extracts of *K. africana* resulted in a significant ($p<0.05$) decrease in lipid parameters when compared with the diabetic control animals (group-2). It can be further stated that *K. africana* plant extracts have the potential to correct the lipid abnormalities, thus delaying lipid peroxidation in diabetic condition.

The reports on the status of antioxidants and antioxidant enzymes in diabetic state are very contradictory; both increase and decrease of antioxidant activity have been reported (Matkovice *et al*., 1982; Kaji *et al*., 1985). The report on the SOD activity in diabetic state is controversial with some authors reporting no change in SOD activity (Kesavulu *et al*., 2000) while others reported increased (Sundaram *et al.,* 1996; Maritin *et al*., 2003) and decreased SOD activity (Sundaram *et al.,* 1996). In the present study, significant ($P<0.05$) decreases in the activities of SOD, CAT and GPx were recorded in diabetic rats not treated compared with the normal control group. An observed significant ($p<0.05$) increases of these antioxidant enzymes were recorded in group 6 treated with a combination of two parts of *K.africana* extracts as against groups 4-5 with monotherpeutic administration of leaf and fruit extracts of the same. Reduction of the antioxidant enzymes activities were observed in diabetic rats not treated with reference to test rats treated with the standard drug. This is in line with the report that products of membrane lipid peroxidation and other oxidants like H_2O_2 may react with superoxide dismutase resulting in oxidative modification thereby causing loss of enzyme activity in diabetic condition (Sundaram *et al.,* 1996). The result also concords with the reports that the relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, pancreatic ß-cells may be vulnerable to reactive oxygen species (ROS) attack when the system is under oxidative stress situation (Lenzen *et al*., 1996; Tiedge et *al*., 1997). Similarly, elevated levels of free radicals, due to the insufficiency of the antioxidant defence system, may lead to disruption of cellular functions, oxidative damages to protein, DNA, membranes and enhance their susceptibility to lipid peroxidation (Baynes, 1999) under uncontrolled diabetic condition. Also, hyperglycemia leads to glycation and inactivation of superoxide dismutase thus attributing to its decrease. In the study, the animals treated with *K. africana* extracts showed an increase in the activity of antioxidant enzymes as against untreated diabetic rats (group 2) and this unveiled the extracts' potential in mopping up or scavenging free radicals generated under oxidative stress- mediated diabetes. The bioactive compound, favonoids may be implicated in the scavenging activity of the plant extracts in oxidative condition.

CONCLUSION

From the results, it can be concluded that 500 mg of *K .africana* extracts possess anti-hyperglycemic effect via α-glycosidase inhibition. Significant reduction of glycol-haemoglobin level and sorbitol concentration in all the diabetic groups treated with either the extracts or reference drug compared with the control groups. The extracts were found to have lipid lowering effects through reduction of total cholesterol, triacyglycerol and low density lipoprotein. *K. africana* increased high-density lipoprotein level probably by decreasing reverse cholesterol transport pathway.

REFERENCES

Ananda, P.K, Kumarappan, C.T., Christudas, S. and Kalaichelvan, V.K. (2012). Effects of *Biophytum sensitivum* on Streptozotocin and nicotinamid-induced diabetic rats. *Asian Pac Journal Tropical Biomedical* **2**(1):31-35.

Aseku, O.T., Olusegun, E. and Adebola, O.(2006). The volatile constituents of the leaves and flower of *Kigelia africana* Benth, *Flva Frangr Journal*, 22 (**1**), 21-23.

Bandawane, D., Juvekar, A. and Juveka, M. (2011). Antidiabetic and streptozotocin-induced diabetic rats. *Indian Journal Pharmacology. Education Research,* **45**(2)**:**114-120.

Baynes, J.W. (1991). Role of oxidative stress in development of complications in diabetes, *Diabtes,* **40**, 405-412.

Frankel, E. (1995). Nutritional benefits of flavonoids. International Conference on Food Factors: Chemistry and Cancer Prevention, Hamamatsu, Japan. *Abstracts*, C6-2.

Gorman, R., Schreiber, L. and Kolodziej, H. (2004). Cuticular wax profile of leaves of some traditionally used African Bignoniaceae, *Z Naturforsch,* **59**: (9-10), 631-635.

Houghton, P.J. (2002). The sausage tree (Kigelia piñata):ethnobotany and recent scientific work, South *Africa Journal Botany*, **68**: (1), 14-20.

Kaji, H., Jurasaki, M. and Ito, K. (1985). Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulindependent) diabetic women. *Klin Wochenschr,* **63**:765-768.

Kesavulu, M.M., Giri, R., Kameswara-Rao, B. and Apparao, C. (2000). Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. *Diabetes Metabolism* **26**:387-392.

Kumar, S., Kumar, V. and Prakash, O. (2011). Antidiabetic and hypolipidemic activities of *Dillenia indica* extract in diabetic rats. *Zhong Xi Yi Jie HE Xue Bao.* **9**(5):570-574.

Kumar, S., Malhotra, R. and Kumar, D. (2010). Antidiabetic and free radicals scavenging potentials of *Euphorbia hirta* flower extract. *Indian Journal Pharmacology Science*. **72** : (4):531-533.

Langerstroer, P. and Pieper, G.M. (1992). Regulation of spontaneous EARR release in diabetic rat aorta by oxygen free radical. *America Journal Physiology*, **263**(32): H251-H265.

Lenzen, S., Tiedge, M., Jorns, A. and Munday, R. (1996). Alloxan derivative as a tool for the elucidation action of alloxan. In: Shafrir E (ed) lessons from animal diabetes. Birkhauser, Boston. pp 113-122.

Maritim, A.C, Sanders R.A, Watkins J.B (2003). Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical Molecular Toxicology* 17:24-38

Matkovics, B., Varga, S .I. Szabó, L. and Witas, H. (1982). The effect of diabetes on the activities of the peroxide metabolic enzymes. *Hormonal Metabolic Research*, **14**:77-79.

Ogugua, V.N., Egba, S.I. and Adoga, J.E (2013). In Vivo and antihyperglycaemic properties of aqeous extract of herbal cocktail. *World Journal of Pharmaceutical Science, 1(1): 1-27*

Ramachandran, V., Mandal, D., Payyavala, U., Sagai, P.D., Muthureddy, N.S. and Shanish, A. (2011). Hypoglycemic activity of *Asparagus racemosus* on streptozotocin-induced diabetic in rats. *Advanced Applied Science Research*. **2**(3):179-185.

Saini, S., Kaur, Verma, B, Ripudaman, and Singh, S.K. (2009). *Kigelia africana* Lam. (Benth.) – An Overview. *Natural Product Radiance,* **8**: (2):190-197.

Sekero, M.R, Sahin H, Dülger H, and Algün E.(2000) .The effect of dietary treatment on erythrocyte lipid peroxidation,superoxide dismutase, glutathione peroxidase, and serum lipid peroxidation in patients with type 2 diabetes mellitus. *Clinical Biochemistry* **33:**669-674.

Shirwaikar, A: Rajendra, and Barik, R; (2006) Effect of aqeous bark extract of Garuga Pinnata Roxb in Streptozotocin – nicotinamide induced type II diabetes. Journal of Ethnopharmacology; **107**:285-290.

Siyem, D., Syngai, G., Khup, P.Z., Khongwir, B.S., Kharbuli, B. and Kayang, H. (2002). Hypogyceamic effects of *Potentilla fulgens L*. in normal and alloxan diabetic mice. *Journal Ethnophymarcology*, **83**:55-61.

Stanely, P., Prince, M. and Menon, V.P.(2000). Hypoglycaemic and other related action of *Tinosporacordifolia* roots in alloxan-induced diabetic rats. *Journal Ethnopharmacology*, **70**(1):9-15.

Suba,V., Murrugesan,T.,Bhaskara, R.R.,Ghosh, L.,Pal, M.,Subhash, C., Mandal, S.C. and Saha, B.P. (2004). Antidiabetic potential of *Barterialupulina* extract in rats. *Fitoterapia*,**7**:1-4.

Sundaram, R.K., Bhaskar, A. and Vijayalingam, S. (1996). Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clinical Science* (Lond) **90**:255-260.

Testamarian, B. and Cohen, R.A.(1992). Free radical mediate endothelial cell dysfunction caused by elevated glucose. *America Journal Physiology*, **263**(32):H321-H326.

Tiedge,M., Lortz, S., Drinkgerm, J. and Lenzen, S.(1997). Relation between antioxidant enzyme gene expression and antioxidant defense system status of insulin producing cells. *Diaebetes*, **46**:1733-1742.

Ting, H.H., Timimi, F.K., Boles, K.S. and Creager, S.J. (1996). Vitamin C improves endothelial cell dependent vasodilatation in patient with non-insulin dependent diabetes mellitus. *Journal of Clinical Investigation*, **97**(1): 22-28.

West, I.C. (2000). Radicals and oxidative stress in diabetes. *Diabetic Medicine*, **17**: 170-180.