

Full Length Research Paper

## Anti-oxidative potentials of some medicinal plants on lipid peroxidation in alloxan-induced diabetic rats

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Diabetes mellitus is a metabolic disease characterized by hyperglycemia and if not properly controlled, culminates in increased oxidative stress and decrease in anti-oxidant levels. The leaves of *Psidium guajava*, *Anacardium occidentale*, *Eucalyptus globulus* and fruits of *Xylopia aethiopica* are used in the management of diabetes mellitus and thus, their anti-oxidative effects in alloxan-induced diabetes mellitus in Wistar albino rats were investigated using standard methods. The administration of *P. guajava*, *A. occidentale*, *E. globulus* leaf and *X. aethiopica* fruit extracts caused significant ( $p < 0.05$ ) increases in the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and vitamin C concentration. These increases in some test groups were not significantly ( $p > 0.05$ ) different from those of the glibenclamide groups, with others better increased than the glibenclamide groups. Malondialdehyde (MDA) concentration was significantly ( $p < 0.05$ ) reduced in all the groups except the diabetic-untreated group that showed a marked increase attesting to the fact that peroxidative activity occurred after the induction of diabetes mellitus. In conclusion, the above plants demonstrated anti-oxidative effects and therefore, may be used in the amelioration of disorders associated with oxidative stress.

**Key words:** Diabetes mellitus, superoxide dismutase, catalase, glutathione.

### INTRODUCTION

Diabetes mellitus is a heterogeneous disease characterized by distorted synthesis and/or secretion of insulin as well as resistance of peripheral tissues to insulin activity. Hyperglycemia (high blood glucose) and persistent or prolonged exposure of body tissues to increased levels of glucose are factors responsible for the development of most diabetic complications (Laurence et al., 2006).

Oxidative stress is defined as a metabolic process, culminating in the production of reactive compounds in the form of free radicals. These are atoms or molecules

that have at least an unpaired electron in their outermost shell or orbital, which are not contributing to molecular bonding and are capable of independent existence (Karlsson, 1997). Amongst such reactive compounds is hydroxyl radical ( $\cdot\text{OH}$ ), the most dangerous member because it is highly reactive and very unstable. Hydroxyl radicals introduce free radical chain reaction, then abstract a hydrogen atom from one of the carbon atoms in the polyunsaturated fatty acids or lipoproteins of plasma membrane of organelles. This results in a biological process known as lipid peroxidation (Devlin,

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**Abbreviations:** SOD, Superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GRx, glutathione reductase; CoQ10, coenzyme Q10; DMSO, dimethyl sulphoxide; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances.

2006). In the event of uncontrolled hyperglycemia, due to poorly managed diabetes, these lipoproteins become oxidized (oxLDL) while their accumulation in the cell membrane results in plasmalemma linking thereby affecting membrane-bound receptors. Meanwhile, the end-product of lipid peroxidation could be mutagenic and carcinogenic, forming many damaging stable aldehydes such as malondialdehyde (propanedial, 4-hydroxy non enal) a biomarker of lipid peroxidation and oxidative stress (Marnett, 1999).

It is of great importance that the rate of production of free radicals and their elimination is in equilibrium. Hence, excessive generation of free radicals and reduction in their elimination from the cells leads to oxidative cellular stress (Valko et al., 2007). However, animal cells have developed a mechanism to counteract the deleterious effect of free radicals, known as antioxidants. Antioxidants are a group of molecules that have the ability to retard or prevent the oxidation of other molecules, by giving up their own electrons to free radicals and becoming oxidized as reducing agents (Sies, 1997).

The defense mechanism of antioxidants are divided into the enzymatic scheme which includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) and the non-enzymatic scheme such as vitamins A, C, E, glutathione, coenzyme Q10 (CoQ10), bioflavonoids, copper, zinc, manganese, selenium, folic acid, albumin, vitamin B1, B2, B6 and B12 (Martim et al., 2003). It is an established fact globally that diabetes is one of the leading causes of death and disability (American Diabetes Association, 2010) with majority of diabetics living in developing countries (King et al., 1998) and increased rate of prevalence found amongst rural population world Wide. This has led to wake-up call for the development of indigenous, inexpensive botanical sources for anti-diabetic (crude or purified) drugs (Venkatesh et al., 2003), that will effectively manage diabetes mellitus and its associated complications which some currently available antidiabetic drugs have failed to manage.

Botanical sources are materials for plant-based drugs, used to treat various diseases for several decades in almost all cultures as medicine (Mushtaq et al., 2009). Medical plants contain secondary metabolites known as phytochemicals that vary significantly in structure. Examples of phytochemicals are saponins, tannins, essential oils, alkaloids, flavonoids, (Harborne, 1973; Sofowora, 1993). They possess curative properties due to their wide range of pharmacological activities or actions (Swain 1966; Trease and Evans, 1989).

These phytochemicals such as alkaloids function in autonomic nervous system and blood vessels (Trease and Evans, 1989; Omotayo and Omoyeni, 2009). Tannins are known to possess antioxidant and antimicrobial properties. Saponins have anticarcinogenic and antidiabetic effects. They are also capable of reducing cholesterol level (Trease and Evans, 1989). Plants such as

*Anacardium occidentale*, *Eucalyptus globulus*, *Psidium guajava* and *Xylopi aethiopic a* have been implicated in the treatment of diabetes mellitus because they are able to lower blood glucose level and possess antioxidant activity.

This present study was aimed at ascertaining the antioxidative potentials of these plants on lipid peroxidation in alloxan-induced diabetic rats.

## MATERIALS AND METHODS

### Preparation of the crude extract

The leaves of *A. occidentale*, *E. globulus*, *P. guajava* and fruits of *X. aethiopic a* were air dried to constant weight at room temperature and then reduced to powder. Six hundred grams of each plant material was macerated in 2.7 l of analytical grade chloroform. After 48 h, the resulting extracts were filtered and concentrated with rotary evaporator at reduced pressure and the yield of extracts calculated.

A standard weight 8 g of each extracts was dissolved in 16 ml of 10% dimethyl sulphuroxide (DMSO) and made up to 25 ml with distilled water. The doses of each extracts administered was estimated by the methods of Tedong et al. (2007), where volumes given were calculated as follows

$$V \text{ (ml)} = \frac{D \times P}{C}$$

Where, D is the dose used (g/kg body weight of test animals); P is the body weight (kg); C is the concentration (g/ml); V is the volume (ml).

### Animals

Fifty (50) male Wistar albino rats of weight (180 -230 g) and 104 male mice of weight (30-40 g) were used for this study. They were housed and maintained at a 12 h light and dark cycle and fed with rat diet *ad libitum*. The mice were used for acute oral toxicity study while the rats were made diabetic by a single dose of 180 mg/kg body weight of alloxan monohydrate intraperitoneally and 44 rats were selected for the study, 72 h after diabetes has been established. Treatments were for 40 h and administrations of the extracts were twice daily. After 40 h, rats were sacrificed and their blood and livers collected for further biochemical analyses.

### Acute oral toxicity test (LD<sub>50</sub>)

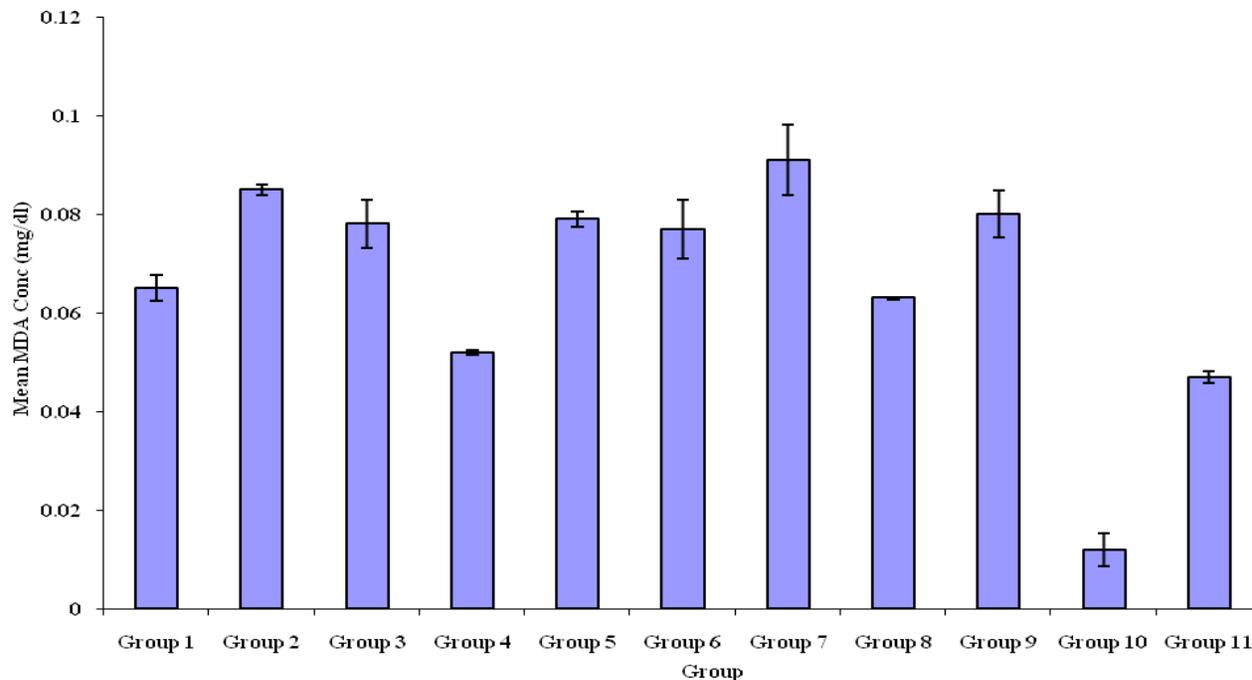
A lethal dose toxicity study of each plant material was carried out by the method described by Lorke (1983).

### Estimation of lipid peroxidation

Lipid peroxidation was determined spectrophotometrically by measuring the level of malondialdehyde (MDA), a lipid peroxidation product, as described by Wallin et al. (1993).

### Evaluation of antioxidant activity

The activity of enzymic antioxidant, catalase was assayed spectrophotometrically based on the methods of Aebi et al. (1983). Glutathione peroxidase activity was assayed by the methods of



**Figure 1.** effect of varying doses of different plant extracts on malondialdehyde (MDA) concentration in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group 5 = *Psidium guajava* (100 mg/kg); Group 6 = *Psidium guajava* (250 mg/kg); Group 7 = *Xylopi aethiopica* (100 mg/kg); Group 8 = *Xylopi aethiopica* (250 mg/kg); Group 9 = Glibenclamide (5 mg/kg); Group 10 = diabetic untreated; Group 11 = DMSO control.

Paglia and Valentine (1967) and Kraus and Ganther (1980). Superoxide dismutase activity was assayed using the methods as described by Wooliams et al. (1983), Suttle (1986) and Arthur and Boyne (1985). The concentration of vitamin C was estimated by the methods of Goodhart and Shils (1973).

#### Statistical analysis

Data generated from this study were represented as mean  $\pm$  SEM. Variables were analyzed by one-way analysis of variance (ANOVA) and comparison done by multiple comparisons using Duncan test.

## RESULTS

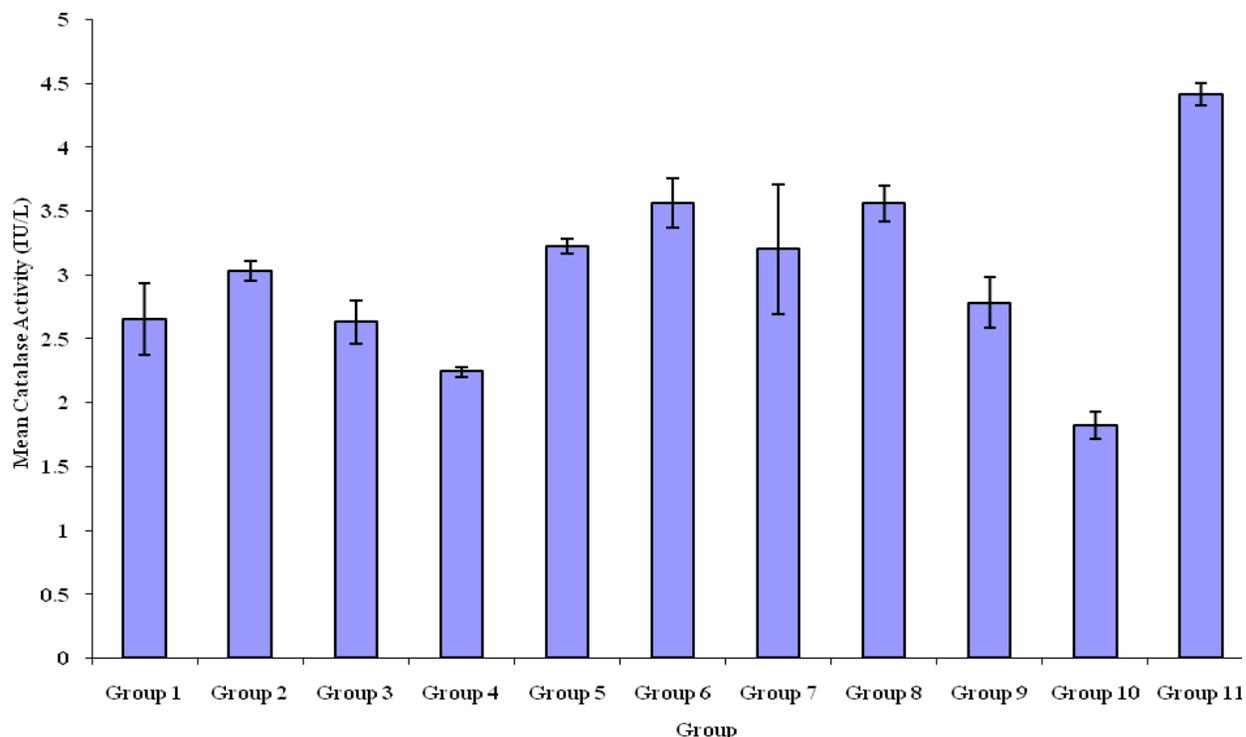
### Effects of varying doses of the different plant extracts on MDA concentration

The data presented in Figure 1 confirm that lipid peroxidative action occurred as seen in increased concentration of MDA in diabetic untreated rats. However, the administration of each extracts at specific dose caused a significant ( $p < 0.05$ ) decrease in MDA concentration of the diabetic rats. The groups administered *E. globulus* (100 and 250 mg/kg) had the greatest reduction in the concentration of MDA while those administered *A. occidentale* (100 and 250 mg/kg) exhibited the second greatest decrease in MDA concentration. Also, the group

treated with *X. aethiopica* had a significantly ( $p < 0.05$ ) reduced MDA concentration. An observable decrease was recorded in the group treated with *P. guajava*. The decrease in MDA concentration of the extract-treated groups were significantly different ( $p < 0.05$ ) when compared to that of the diabetic untreated group, with reductions prominent mostly with the 250 mg/kg groups. However, there was no significant ( $p > 0.05$ ) difference in the MDA concentrations of the 100 mg/kg groups of all the extracts compared with the glibenclamide (5 mg/kg) group.

### Effects of varying doses of the different plant extracts on CAT activity

Figure 2 shows that DMSO group had the highest activity of CAT but the diabetes mellitus reduced this antioxidant activity. However, the administration of the various extracts led to a significant ( $p < 0.05$ ) increase in the catalase activity especially the 250 mg/kg groups of each extract. *P. guajava* (100 and 250 mg/kg) had the greatest increase in CAT activity and *E. globulus* (100 and 250 mg/kg) showed the second highest increase. *A. occidentale* (100 and 250 mg/kg) significantly ( $p < 0.05$ ) increased CAT activity as shown. Also, the *X. aethiopica* treatment group had a remarkable rise. The increase of



**Figure 2.** effect of varying doses of different plant extracts on catalase (CAT) concentration in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group 5 = *Psidium guajava* (100 mg/kg); Group 6 = *Psidium guajava* (250 mg/kg); Group 7 = *Xylopi aethiopica* (100 mg/kg); Group 8 = *Xylopi aethiopica* (250 mg/kg); Group 9 = Glibenclamide (5 mg/kg); Group 10 = diabetic untreated; Group 11 = DMSO control.

CAT activity in the glibenclamide group was not significantly ( $p > 0.05$ ) different from that of the 100 mg/kg groups of the extracts.

#### Effects of varying doses of the different plant extracts on GPx activity

Figure 3 shows that *A. occidentale* treatment group had the highest increase in glutathione peroxidase (GPx) activity which was significant ( $p < 0.05$ ) compared with diabetic untreated group. In a descending order, significant ( $p < 0.05$ ) increases occurred in the groups administered *P. guajava* (100 and 250 mg/kg), *E. globulus* (100 and 250 mg/kg) and *X. aethiopica* (100 mg/kg). There was no significant ( $p > 0.05$ ) difference in the GPx activity of glibenclamide (5 mg/kg) group compared to those of *A. occidentale* (100 and 250 mg/kg) and *P. guajava* (100 mg/kg).

#### Effects of varying doses of the different plant extracts on SOD activity

Figure 4 represents the effect of the plant extracts on superoxide dismutase (SOD) activity at different doses in

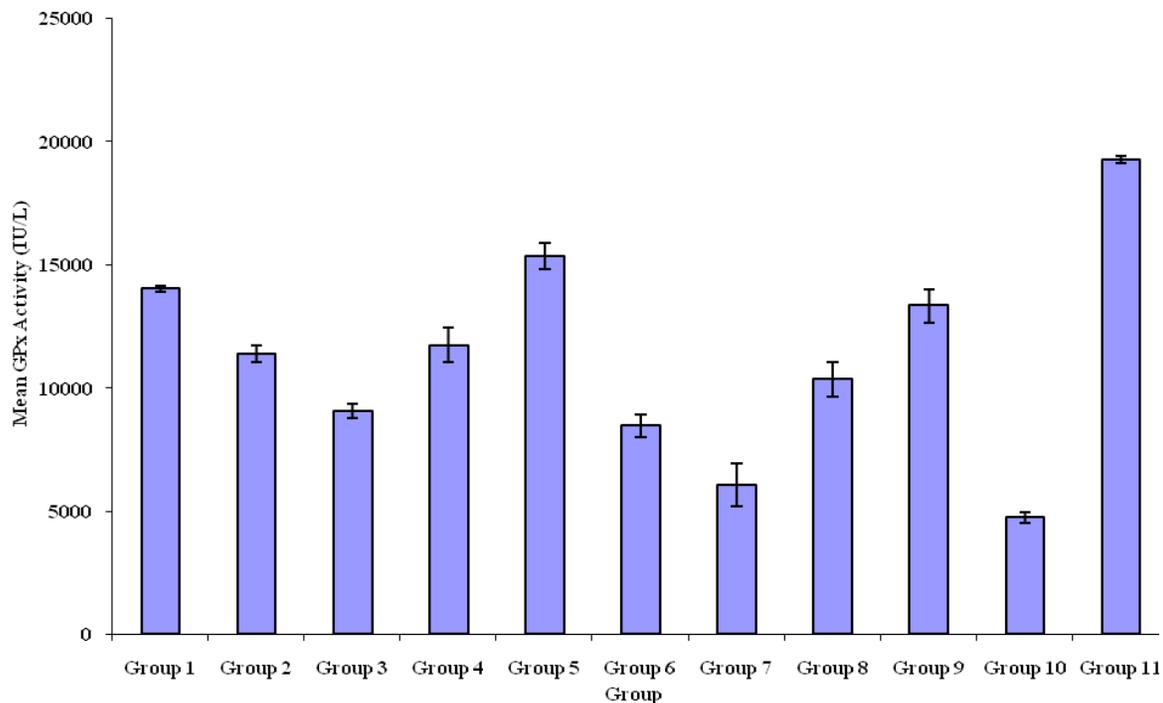
the diabetic rats. *A. occidentale* (100 and 250 mg/kg) groups showed a significant ( $p < 0.05$ ) increase in SOD activity when compared to those of *P. guajava* (100 and 250 mg/kg), *E. globulus* (100 mg/kg), *X. aethiopica* (100 and 250 mg/kg) and glibenclamide. Moreover, a notable difference was observed between the diabetic untreated groups and other test groups.

#### Effects of varying doses of the different plant extracts on vitamin C activity

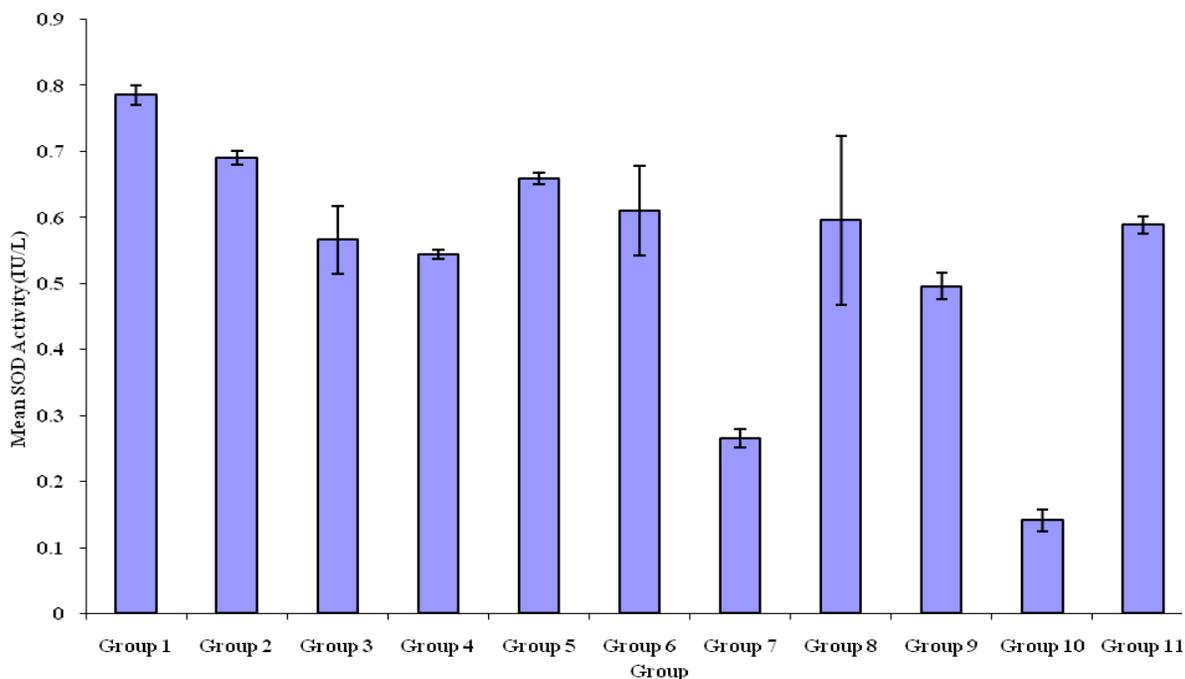
Figure 5 shows the effect of the extracts on vitamin C activity in rats. The administration of the extracts resulted in significant ( $p < 0.05$ ) increases in this antioxidant concentration. The highest increase was recorded in the *P. guajava* (100 and 250 mg/kg) groups compared to those of *X. aethiopica* (100 and 250 mg/kg), *A. occidentale* (100 and 250 mg/kg), *E. globulus* (100 and 250 mg/kg) and glibenclamide. A significant ( $p < 0.05$ ) difference was observed between every test group and diabetic untreated group.

## DISCUSSION

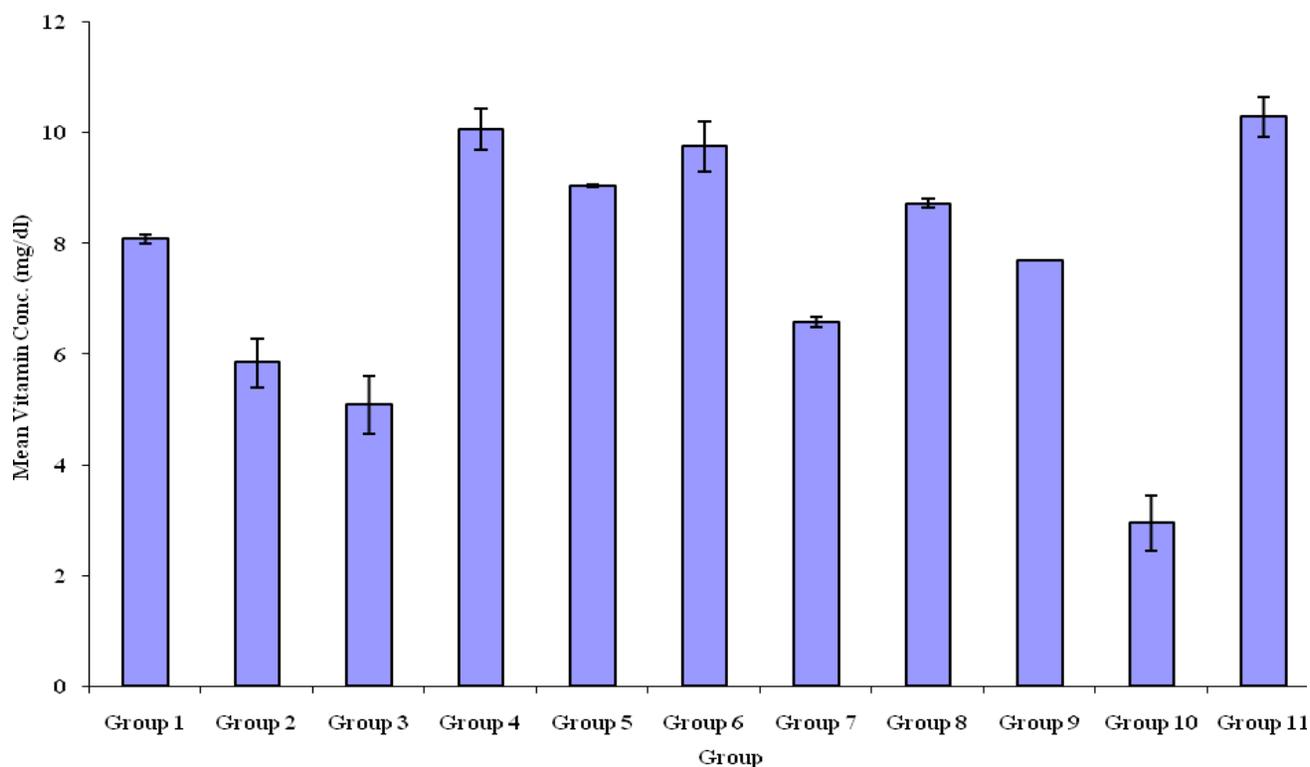
The data in our present study shows the relationship



**Figure 3.** effect of varying doses of different plant extracts on glutathione peroxidase (GPx) activity in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group 5 = *Psidium guajava* (100 mg/kg); Group 6 = *Psidium guajava* (250 mg/kg); Group 7 = *Xylopi aethiopica* (100 mg/kg); Group 8 = *Xylopi aethiopica* (250 mg/kg); Group 9 = Glibenclamide (5 mg/kg); Group 10 = diabetic untreated; Group 11 = DMSO control.



**Figure 4.** Effect of varying doses of different plant extracts on superoxide dismutase (CAT) activity in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group 5 = *Psidium guajava* (100 mg/kg); Group 6 = *Psidium guajava* (250 mg/kg); Group 7 = *Xylopi aethiopica* (100 mg/kg); Group 8 = *Xylopi aethiopica* (250 mg/kg); Group 9 = Glibenclamide (5 mg/kg); Group 10 = diabetic untreated; Group 11 = DMSO control.



**Figure 5.** Effect of varying doses of different plant extracts on vitamin activity in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group 5 = *Psidium guajava* (100 mg/kg); Group 6 = *Psidium guajava* (250 mg/kg); Group 7 = *Xylopi aethiopica* (100 mg/kg); Group 8 = *Xylopi aethiopica* (250 mg/kg); Group 9 = Glibenclamide (5 mg/kg); Group 10 = diabetic untreated; Group 11 = DMSO Control.

between diabetes mellitus and lipid peroxidation, where hyperglycemia, a typical characteristic of diabetes mellitus triggers the generation of free radicals. In turn, oxidative stress causes peroxidation of plasma lipid membrane of tissues. From our study, *P. guajava* performed best in overcoming the peroxidative effect of the free radicals, judging from its ability to improve the antioxidant activities/concentration of the test rats. This is supported by the work of Sowmya et al. (2010) which showed that *P. guajava* reduced thiobarbituric acid reactive substances (TBARS), increased the activities of superoxide dismutase and glutathione reductase as well as the glutathione level in the cardiac tissues of diabetic rats.

*A. occidentale* from our results also increased the antioxidant activity/concentration and reduced lipid peroxidation product (MDA) significantly, further confirming the work of Mary (2004) on the interaction of *A. occidentale* leaf extract with standard blood glucose measurement (GOD-PAP) which resulted in the under estimation of the actual glucose concentration indicating that the extract possesses a high reducing power, a potential antioxidant property.

Also, our results show that *E. globulus* possesses antioxidant ability by increasing the activity/concentration

of CAT, GPx, SOD and vitamin C. This plant performed best at reducing MDA concentration of the diabetic rats. This effect is validated by Alireza et al. (2009) who studied the attenuation of oxidative stress of streptozotocin-induced diabetic rats by *E. globulus*, whereby it decreased the concentrations of the liver MDA, glycosylated hemoglobin (HbA<sub>1c</sub>) and TBARS significantly.

*X. aethiopica* also significantly reduced the MDA concentration in diabetic rats and increased the antioxidant activity/concentration of CAT, GPx, SOD and vitamin C. The performance of this extract is authenticated by Adaramoye et al. (2011) who reported that the synergistic treatment of *X. aethiopica* and vitamin C attenuated the adverse effects of irradiation on liver glutathione-S-transferase (GS) and catalase activities after a week of exposure and liver SOD and kidney catalase after eight weeks of irradiation. The antioxidative effects of the extracts these plants may be attributed to their phytochemical constituents.

In conclusion, the leaves of *A. occidentale*, *E. globulus*, *P. guajava* and fruits of *X. aethiopica* demonstrated antioxidative effects and therefore, may be used in the amelioration of disorders associated with oxidative stress. Also the plants used in this study performed better than

the standard antidiabetic drug (glibenclamide) in reducing lipid peroxidation and enhancing the antioxidant status of the test rats.

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