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Full Length Research Paper

Synergic effects of some medicinal plants on anti-oxidant status and lipid peroxidation in diabetic rats

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The leaves of *Psidium guajava, Anacardium occidentale, Eucalyptus globulus* and fruits of *Xylopia aethiopica* are used in the management of diabetes mellitus. Hence, the phytochemical constituents as well as the acute toxicity of the combined chloroform extracts (*A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica*) and their effects (at graded doses of 100 and 250 mg/kg body weight each) on the concentration of malondialdahyde (MDA), activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and concentration of vitamin C in diabetic and normal rats were investigated using standard methods. The phytochemical analyses of the four extracts showed the presence of terpenoids and fats and oil in all of them. Each of the combined extract was found to be non-toxic even at a dose as high as 5000 mg/kg body weight. The combined extracts at the tested doses significantly ($p < 0.05$) and dose-relatedly reduced the concentration of MDA, raised the activities of CAT, GPx and SOD as well as the concentration of vitamin C in the treated rats. The effects of the combined extracts (especially 250 mg/kg body weight of *A. occidentale* + *E. globulus*) were better than that of the standard anti-diabetic drug [glibenclamide (5 mg/kg body weight)]. The data of this investigation imply that the combined chloroform extracts of the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* may be preferentially used in the management and/or amelioration of diabetes mellitus and its associated complications.

Key words: Anti-oxidants, diabetes mellitus, lipid peroxidation, *Anacardium occidentale*, *Eucalyptus globulus*, *Psidium guajava* and *Xylopia aethiopica*.

INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and/or decreased responsiveness of the pancreas to secreted insulin (Matsui et al., 2007). The several forms of this disease are characterized by persistent or recurrent hyperglycemia that provokes free radical production through the autoxidation of glucose, leucocyte activation and increased transition metal bioavailability (Lai, 2008). Pancreatic beta cells are susceptible to reactive oxygen species (ROS) attack causing beta cell dysfunction that affects insulin production and culminates in a large degree of oxidative damage typified by elevated levels of lipid (malondialdahyde) and DNA peroxidation. This impairs endogenous anti-oxidant defense systems and plays a major role in the pathogenesis and development of diabetic complications. The susceptibility of beta cells to ROS attack is due to their reduced levels of free radical-scavenging anti-oxidant enzymes. Endogenous anti-oxidant defense mechanism/strategy involves the enzymatic and the non-enzymatic systems which functions are to equipoise the toxic effects of free radicals. The enzymatic anti-oxidants are: glutathione peroxidase

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(GPx), glutathione reductase (GRx), catalase (CAT), superoxide dismutase (SOD), coenzyme Q10 (CoQ10). The non-enzymatic anti-oxidants are mixed vitamins A, C, D and E, carotenoids, various bioflavonoids, copper, zinc, manganese, selenium, folic acid, uric acid, albumin, vitamins B_1, B_2 and B_6 (Maritim et al., 2003).

Currently, the available drugs used in the management of diabetes mellitus have not been able to effectively control diabetic complications. Therefore, a shift to alternative source using plants stands to provide a possible anti-hyperglycemic agent that can delay diabetic complications or repair abnormal metabolic pathways/ mechanisms. Medicinal plants contain secondary metabolites known as phytochemicals that vary significantly in structure. Examples of phytochemicals are saponins, tannins, essentials oils, alkaloids and flavonoids. They possess curative properties due to their wide range of pharmacological activities or actions (Trease and Evans, 1989). These phytochemicals such as alkaloids function in autonomic nervous system and blood vessels. Tannins are known to possess anti-oxidant and anti-microbial properties. Saponins have anti-carcinogenic and anti-diabetic effects. They are also capable of reducing cholesterol level (Omotayo and Omoyeni, 2009). Plants such as *Anacardium occidentale, Eucalyptus globulus, Psidium guajava* and *Xylopia aethiopica* have been implicated in the treatment of diabetes mellitus because they possess anti-oxidant activity and are able to lower blood glucose concentration. Thus, this present study was aimed at investigating the combined effects of the chloroform extracts of the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* on anti-oxidant status and lipid peroxidation in diabetic rats.

MATERIALS AND METHODS

The plant samples

The leaves of *A. occidentale*, *E. globulus* and *P. guajava* were collected from the premises of University of Nigeria, Nsukka while the fruits of *X. aethiopica* were purchased from a local market in Delta State. The plant samples were identified by Prof. (Mrs.) May Nwosu of the Department of Botany, University of Nigeria, Nsukka where the voucher specimens were deposited in the herbarium.

Preparation of the extracts

The leaves of *A. occidentale, E. globulus, P. guajava* and the fruits of *X. aethiopica* 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 liters 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 of the two proportionally combined extracts was dissolved in 16 ml of 10% dimethyl sulphuroxide (DMSO). The doses of each extracts administered was estimated by the method of Tedong et al. (2007), where volumes given were calculated as follows:

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

Where D = Dose used (g/kg body weight of test animals), P = body weight (kg), C = concentration (g/ml) and V = volume (ml).

Animals

Thirty-five male Wistar albino rats of weight (180 to 230 g) and sixty-four male mice of weight (20 to 30 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 intraperitonially and thirty-five rats selected for the study, 72 h after diabetes has been established. Treatments were for 40 h and administrations of the combined extracts were twice daily. After 40 h, rats were sacrificed and their blood and livers collected for further biochemical analyses.

Chemicals and reagents

Dimethyl sulfoxide [DMSO (Serva Heidelberg, New York)], chloroform (Sigma Aldrich Chemicals, Germany), alloxan monohydrate (Sigma Aldrich Chemicals, Germany). thiobarbituric acid (Lab Tech Chemical, Avishkar), trichloroacetic acid (Mayer and Baker, England), sodium chloride, cupric acid salts (Mayer and Baker, England), dilute tetraoxosulphate (vi) acid, 2% (v/v) hydrochloric acid, 1% (w/v) picric acid, methyl orange, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Fehling's solution, 5% (w/v) ferric chloride solution, aluminium chloride solution, lead subacetate solution, ammonium solution and distilled water were used. The commercial kits were purchased from Randox Laboratories Ltd, Crumlin Co Antrim, UK.

Phytochemical analyses

Qualitative phytochemical analyses were carried out on the extracts of the various plant samples according to the procedures outlined by Harborne (1998) and Trease and Evans (1989).

Acute oral toxicity test (LD_{50})

A lethal dose toxicity study of each of the two proportionally combined extracts 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 the activities and/or concentration of the anti-oxidants

The activity of catalase was assayed spectrophotometrically based on the methods of Aebi et al. (1983). Glutathione peroxidase activity was assayed by the methods of Paglia and Valentine (1967) and Kraus and Ganther (1980). Superoxide dismutase activity was assayed using the methods as described by Wooliams et al. (1983),

Table 1. Qualitative phytochemical constituents of *Anacardium occidentale*, *Eucalyptus globulus*, *Psidium guajava* and *Xylopia aethiopica.*

Phytochemical constituents	*Anacardium occidentale*	*Eucalyptus Globulus*	*Psidium guajava*	*Xylopia Aethiopica*
Alkaloids	+	+	ND	+
Flavonoids	+	+	+	+
Glycosides	ND	+	+	+
Saponins	+	+	+	ND
Tannins	+	+	+	ND
Terpenoids	+	+	+	+
Fats and oil	+	+	+	+

+ = Present and ND = Not detected.

Sutlle (1986) and Arthur and Boyne (1985). The concentration of vitamin C was estimated by the method of Goodhart and Shils (1973).

Statistical analysis

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

RESULTS

Qualitative Phytochemical Constituents of the Chloroform Extracts of the Leaves of *Anacardium occidentale*, *Eucalyptus globulus* and *Psidium guajava* and the Fruits of *Xylopia aethiopica*

The qualitative phytochemical analyses showed the presence of terpenoids and fats and oil in the four extracts (Table 1). Flavonoids, saponins and tannins were present in the extracts of *A. occidentale*, *E. globulus* and *P.* guajava. Glycosides and alkaloids were not detected in the extracts of *A. occidentale* and *P. guajava*, respectively. Saponins and tannins were not detected in *X. aethiopica* extract.

The acute toxicity and lethality (LD_{50}) of the combined plant extracts

There was no lethality or any sign of toxicity in the four groups of four mice each that received 10, 100 and 1000 mg/kg body weight of each of *A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica* as well as 5 ml/kg body weight of 10% DMSO, respectively at the end of the first phase of the study. At the end of the second phase of the study, there was neither death nor obvious sign of toxicity in the groups of mice that received 1900, 2600 and 5000 mg/kg body weight of each of the combined plant extracts.

Effects of varying doses of the combined plant extracts on malondialdehyde (MDA) concentration

Figure 1 shows that the diabetic untreated group had the highest concentration of MDA but administration of the different doses of the combined extracts resulted in significant ($p < 0.05$) decrease in MDA concentration. The 100 and 250 mg/kg body weight of each of *A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica* in a similar manner as the standard anti-diabetic drug [glibenclamide (5 mg/kg body weight)] significantly ($p < 0.05$) and dose-dependently reduced the MDA concentration when compared with the value obtained for the diabetic untreated group. However, the 250 mg/kg body weight of *A. occidentale* + *E. globulus* caused the greatest reduction in the MDA concentration.

Effects of varying doses of the combined plant extracts on catalase (CAT) activity

The CAT activities of groups 2, 3, 4 and 5 were significantly ($p < 0.05$) higher than that of the diabetic untreated group (group 6). There was however no significant ($p > 0.05$) increase in the CAT activity of group 1 when compared with that of the group 6. There were also non-significant ($p>0.05$) differences between the CAT activities of groups 2, 3 and 4 and that of the group 7 [DMSO control group (5 ml/kg body weight)] as shown in Figure 2.

Effects of varying doses of the combined plant extracts on glutathione peroxidase (GPx) activity

As shown in Figure 3, the diabetic untreated group had the lowest GPx activity but administration of the different doses of the combined extracts caused significant ($p < 0.05$) increase in GPx activity. The 100 and 250 mg/kg body weight of each of *A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica* in a similar manner as the standard anti-diabetic drug [glibenclamide (5 mg/kg body

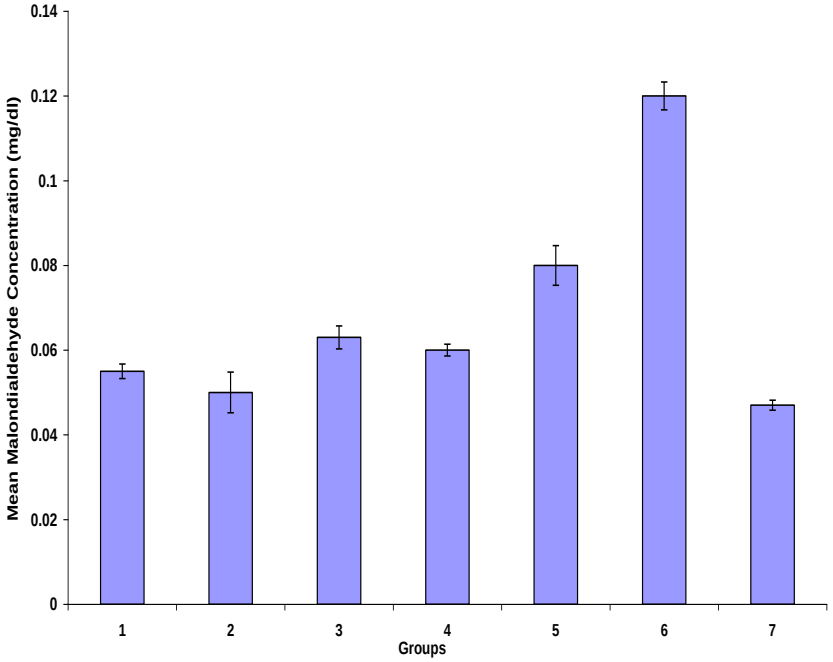

Figure 1. Effects of varying doses of the combined plant extracts on malondialdehyde (MDA) concentration. Group 1 = *A. occidentale* + *E. globulus* (100 mg/kg b.w), Group 2 = *A. occidentale* + *E.. globulus* (250 mg/kg b.w), Group 3 = *P. guajava* + *X.. aethiopica* (100 mg/kg b.w), Group 4 = *P. guajava* + *X. aethiopica* (250 mg/kg b.w), Group 5 = Glibenclamide (5 mg/kg b.w), Group 6 = Diabetic Untreated, Group 7 = DMSO Control (5 ml/kg b.w).

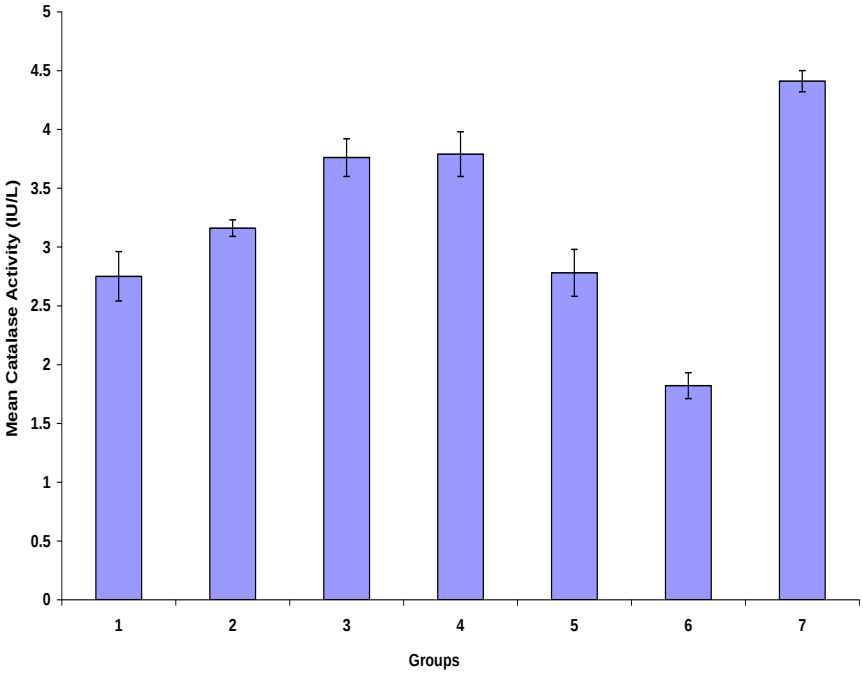

Figure 2. Effects of varying doses of the combined plant extracts on catalase activity. Group 1= *A. occidentale* + *E. globulus* (100 mg/kg b.w), Group 2= *A. occidentale* + *E. globulus* (250 mg/kg b.w), Group 3= *P. guajava* + *X.. aethiopica* (100 mg/kg b.w), Group 4 = *P. guajava* + *X. aethiopica* (250 mg/kg b.w), Group 5 = Glibenclamide (5 mg/kg b.w), Group 6 = Diabetic Untreated, Group 7 = DMSO Control (5 ml/kg b.w).

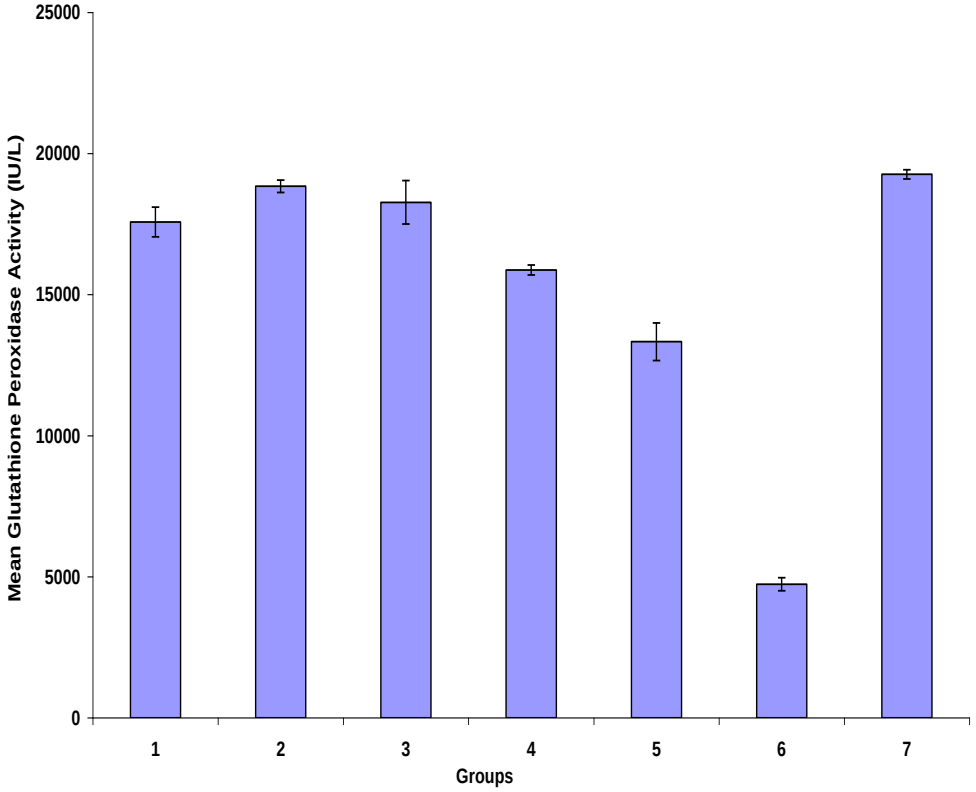

Figure 3. Effects of varying doses of the combined plant extracts on glutathione peroxidase (GPx) activity. Group 1= *A. occidentale* + *E. globulus* (100 mg/kg b.w), Group 2= *A. occidentale* + *E. globulus* (250 mg/kg b.w), Group 3 = *P. guajava* + *X.. aethiopica* (100 mg/kg b.w), Group 4 = *P. guajava* + *X. aethiopica* (250 mg/kg b.w), Group 5 = Glibenclamide (5 mg/kg b.w), Group 6 = Diabetic Untreated, Group 7 = DMSO control (5 ml/kg b.w).

weight)] significantly ($p < 0.05$) raised the activities of GPx when compared with the value obtained for the diabetic untreated group. The 250 mg/kg body weight of *A. occidentale* + *E. globulus* however, elevated the activity of GPx best.

Effects of Varying Doses of the Combined Plant Extracts on Superoxide Dismutase (SOD) Activity

The SOD activities of groups 1, 2, 3, 4 and 5 were significantly ($p < 0.05$) and dose-dependently greater than that of the diabetic untreated group (group 6). There were nevertheless, non-significant ($p > 0.05$) differences between the SOD activities of groups 1, 2, 3, 4 and 5 and that of the group 7 [DMSO control group (5 ml/kg body weight)] as shown in Figure 4.

Effects of varying doses of the combined plant extracts on vitamin C concentration

As shown in Figure 5, the diabetic untreated group had the most reduced concentration of vitamin C but administration of the different doses of the combined extracts caused significant ($p < 0.05$) increase in the concentration of vitamin C. The 100 and 250 mg/kg body weight of each of *A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica* in a similar manner as the standard anti-diabetic drug [glibenclamide (5 mg/kg body weight)] significantly ($p<0.05$) and dose-dependently increased the concentrations of vitamin C when compared with the value obtained for the diabetic untreated group.

DISCUSSION

Acute toxicity test on the combined extracts (*A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica*) using mice showed an LD_{50} value of greater than 5000 mg/kg body weight for each combined extract which indicates that the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* might be regarded as being safe with remote risk of acute toxicity. The 250 mg/kg body weight of the combined extract (*P. guajava* + *X. aethiopica*) exerted better effects in the treated rats than all the doses of *A. occidentale* + *E. globulus*

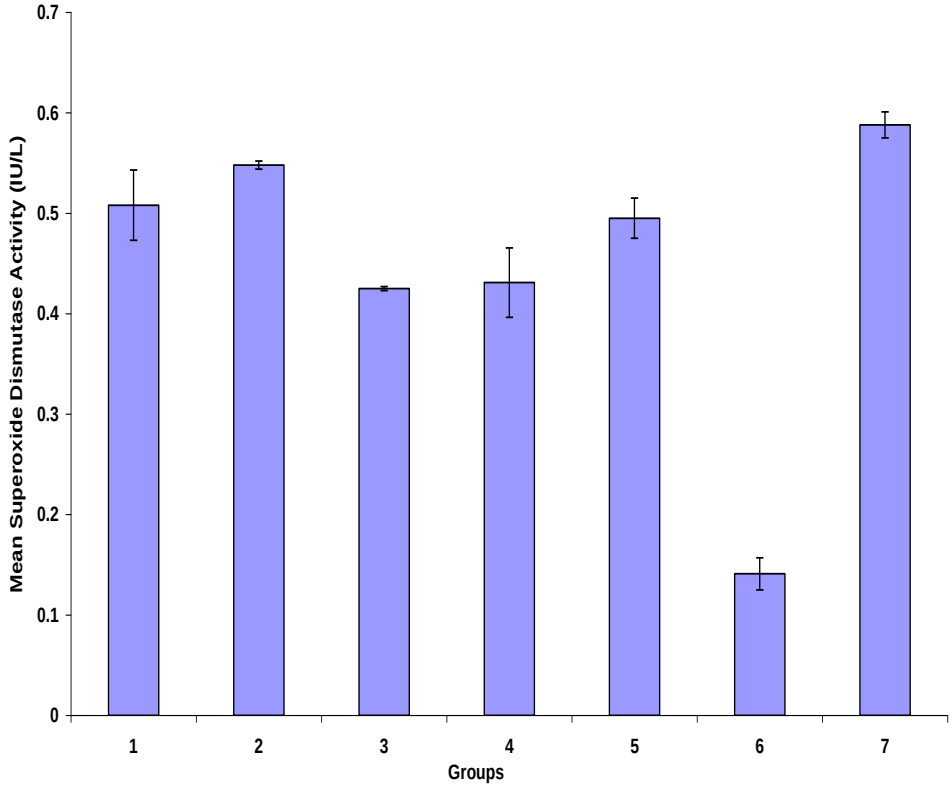

Figure 4. Effects of varying doses of the combined plant extracts on glutathione peroxidase (GPx) activity. Group 1= *A. occidentale* + *E. globulus* (100 mg/kg b.w), Group 2 = *A. occidentale* + *E. globulus* (250 mg/kg b.w), Group 3= *P. guajava* + *X.. aethiopica* (100 mg/kg b.w), Group 4 = *P. guajava* + *X. aethiopica* (250 mg/kg b.w), Group 5 = Glibenclamide (5 mg/kg b.w), Group 6 = Diabetic Untreated, Group 7 = DMSO Control (5 ml/kg b.w).

considering all the parameters studied. The ability of *A. occidentale* + *E. globulus* to reduce the concentration of MDA and increase the activities of GPx and SOD better than *P. guajava* + *X. aethiopica* in the present study might be attributed to the presence of saponins, flavonoids and alkaloids in the extracts of *A. occidentale* and *E. globulus* as revealed by the result of the qualitative phytochemical analyses. Saponins possess anti-carcinogenic property against MDA that could be carcinogenic and mutagenic (Marnett, 1999).

Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive anti-diabetic principles (Yasir et al., 2012). These performances of the combined extracts are further supported by the work of Hayashi et al. (2001) who reported that the oral administration of *E. globulus* to diabetic rats culminated in the elevation of SOD and GPx activities in the liver and kidney and reduced lipid peroxidation in these organs. Also, Pinto et al. (2007) reported that the anti-diabetic activity of *E. globulus* was connected to the presence of high manganese (anti-oxidant mineral) content of the plant which was responsible for the anti-oxidant property in both the individual and the synergic study groups. Furthermore, Sowmya et al. (2010) showed that the administration of ethyl acetate fraction of *P. guajava* leaves to streptozotocin-induced diabetic rats caused significant ($p<0.05$) decrease in lipid peroxidation and glycation products as well as elevated anti-oxidant in a dose-dependent manner.

Adaramoye et al. (2011) reported that the synergic treatment of *X. aethiopica* and vitamin C significantly ($p < 0.05$) decreased the levels of lipid peroxidation in serum, kidney and liver of irradiated animals. This was probably attributed to the increase in the expression of mRNA for γ-glutamylcysteine synthase, a rate-limiting enzyme in the synthesis of GSH.

Conclusion

The data of this investigation show that the combined chloroform extracts of the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* performed better in all the parameters determined than glibenclamide (a standard anti-diabetic drug) and therefore, could be preferentially used in the management and/or amelioration of diabetes mellitus and its associated complications.

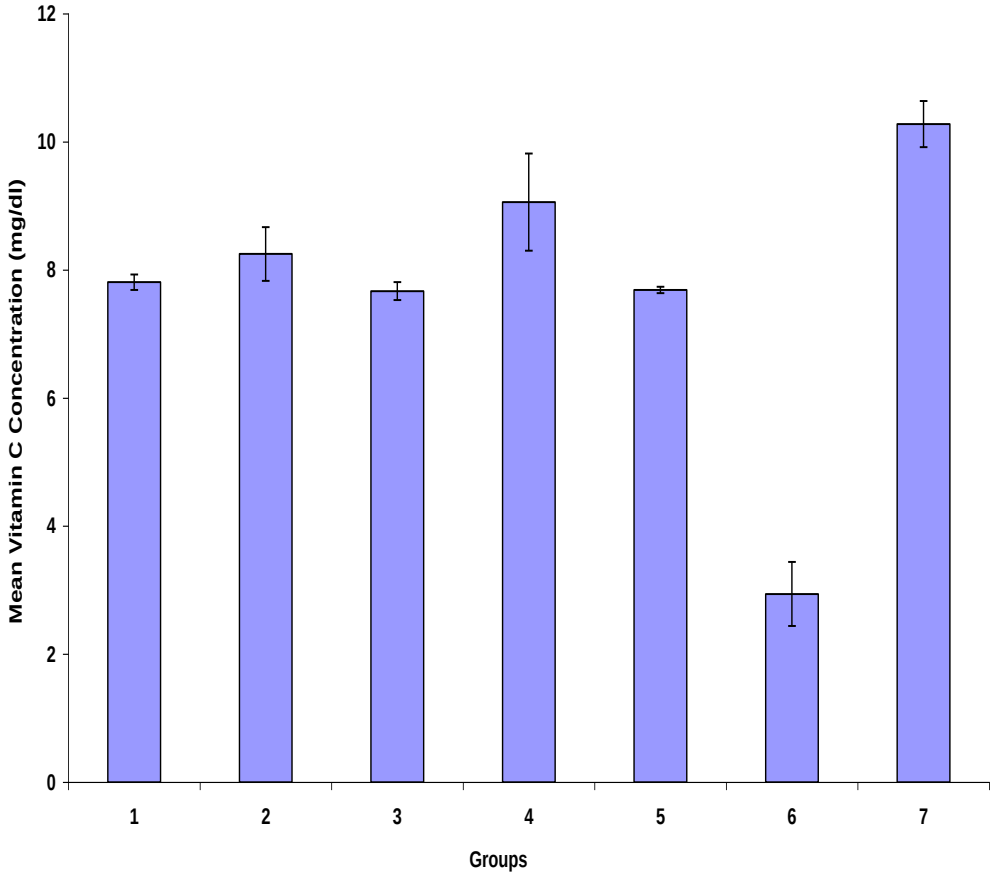

Figure 5. Effects of varying doses of the combined plant extracts on vitamin C concentration. Group 1 = *A. occidentale* + *E. globulus* (100 mg/kg b.w), Group 2 = *A. occidentale* + *E. globulus* (250 mg/kg b.w), Group 3 = *P. guajava* + *X.. aethiopica* (100 mg/kg b.w), Group 4 = *P. guajava* + *X. aethiopica* (250 mg/kg b.w), Group 5 = Glibenclamide (5 mg/kg b.w), Group 6 = Diabetic Untreated, Group 7 = DMSO Control (5 ml/kg b.w).

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