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Full Length Research Paper

Collaborative effects of some anti-diabetic plants on the liver marker enzymes of 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. 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 activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were therefore, assayed in diabetic and normal rats using standard methods. The phytochemical analyses of the four extracts showed the presence of flavonoids, 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-dependently decreased the activities of ALT, AST and ALP. The effects of the combined extracts (especially 250 mg/kg body weight of *P. guajava* + *X. aethiopica*) were better than that of the standard anti-diabetic drug [glibenclamide (5 mg/kg body weight)]. The results generally indicate that the combined chloroform extracts of the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* might be adopted for the management and/or amelioration of diabetes mellitus and its accompanying complications.

Key words: *Psidium guajava, Anacardium occidentale, Eucalyptus globules*, *Xylopia aethiopica,* chloroform extracts

INTRODUCTION

Diabetes mellitus is a heterogenous metabolic disorder characterized by hyperglycemia arising from defective insulin secretion, resistance to insulin action or both. This disease generally affects organs that participate in carbohydrate metabolism such as the liver, a major site of insulin clearance and glucose homeostasis during the concentration of postprandial fasting blood glucose (Gavin et al., 1997). The toxicity of excessively high concentration of plasma glucose arising from prolonged hyperglycemia or postprandial glucose cause the autoxidation of glucose and non-enzymatic glycosylation of proteins leading to the production of advanced glycolsylation end products (AGEs) which through their receptors (RAGEs) inactivate enzymes, altering their structures and functions and resulting in free radical formation (McCarthy et al., 2001).

The free radicals through oxidative stress cause lipid peroxidation resulting in cell injury and decreased concentration of cellular anti-oxidants (Hijora et al., 2000). Malondialdehyde (MDA), a product of lipid peroxidation, if elevated in the blood can lead to activation of leucocyte lysosomal membrane rupture releasing the enzymes into general circulation. These results in the increase of cell and parenchymal tissue damage for example, liver cell paren-

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chymal destructions causing liver enzymes to leak into the blood stream and this serves as an indicator for free radial-mediated destruction of liver parenchymal cells (Pratibha et al., 2004). Plants such as *Anacardium occidentale, Eucalyptus globulus, Psidium guajava,* and *Xylopia aethiopica* have been implicated in the treatment of diabetes mellitus because they possess arrays of phytochemicals with multifarious pharmacological effects (Sagheb et al., 2010; Gupta et al., 2011; Ukwenya et al., 2012). Hence, the present study was targeted at evaluating the combined effects of the chloroform extracts of the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* on liver marker enzymes of 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. 600 g 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 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); V = Volume (ml)

Animals

Thirty-five male Wistar albino rats of weight (180-230 g) and 64 male mice of weight (20-30 g) were used for this study. The University Animal Research Ethical Committee approved the experimental protocol. The animals 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 35 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 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), sodium chloride, 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 for the study. The commercial kits were purchases 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).

Experimental procedures

Alanine and aspartate aminotransferase (ALT and AST) activities were assayed using Randox commercial enzyme kit as described by Reitman and Frankel (1957) and Schmidt and Schmidt (1963). Alkaline phosphatase (ALP) activity was estimated using Randox commercial enzyme kit, based on the methods of Rec (1972) and Englehardt (1970).

Statistical analysis

Data generated from this study were represented as 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 *A. occidentale*, *E. globulus* and *P. guajava* and the fruits of *X. aethiopica*

The qualitative phytochemical analyses showed the presence of flavonoids, terpenoids and fats and oil in the four extracts (Table 1). 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

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

Phytochemical constituent	*Anacardium occidentale*	*Eucalyptus globulus*	*Psidium guajava*	*Xylopia aethiopica*
Alkaloids	+	+	ND	+
Flavonoids	+	+	+	+
Glycosides	ND	+	+	+
Saponins	+	+	+	ND
Tannins	+	+	+	ND
Terpenoids	+	+	+	+
Fats and oil	+	+	+	+

+ = Present; ND = Not detected.

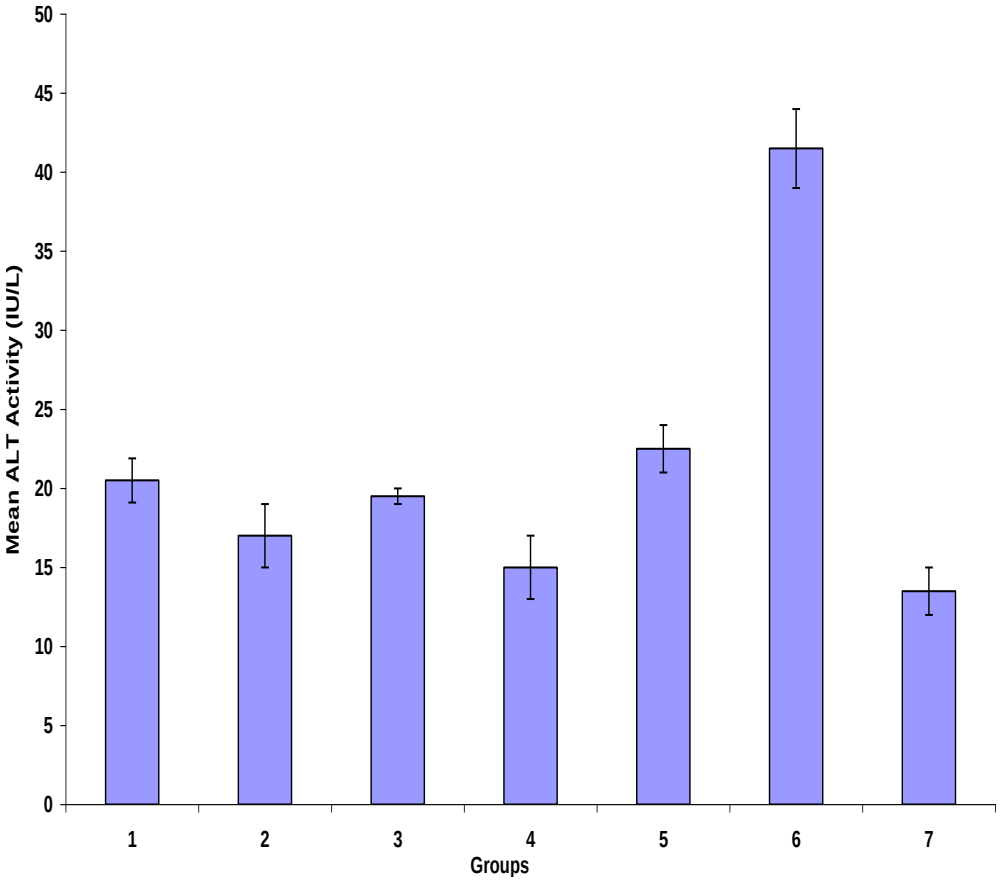

Figure 1. Effects of varying doses of the combined plant extracts on alanine aminotransferase (ALT) activity. Group 1= *A. occidentale* + *E. globulus* (100 mg/kg b.w). Group 2 = *A. occidentale* + *E. globulus* (250 mg/kg b.w). Group 3 = *P. guajava* + *X. aethiopica* (100 mg/kg b.w). Group 4 = *P.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

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 alanine aminotransferase (ALT) activity

Figure 1 shows that the diabetic untreated group had the highest activity of ALT but administration of the different

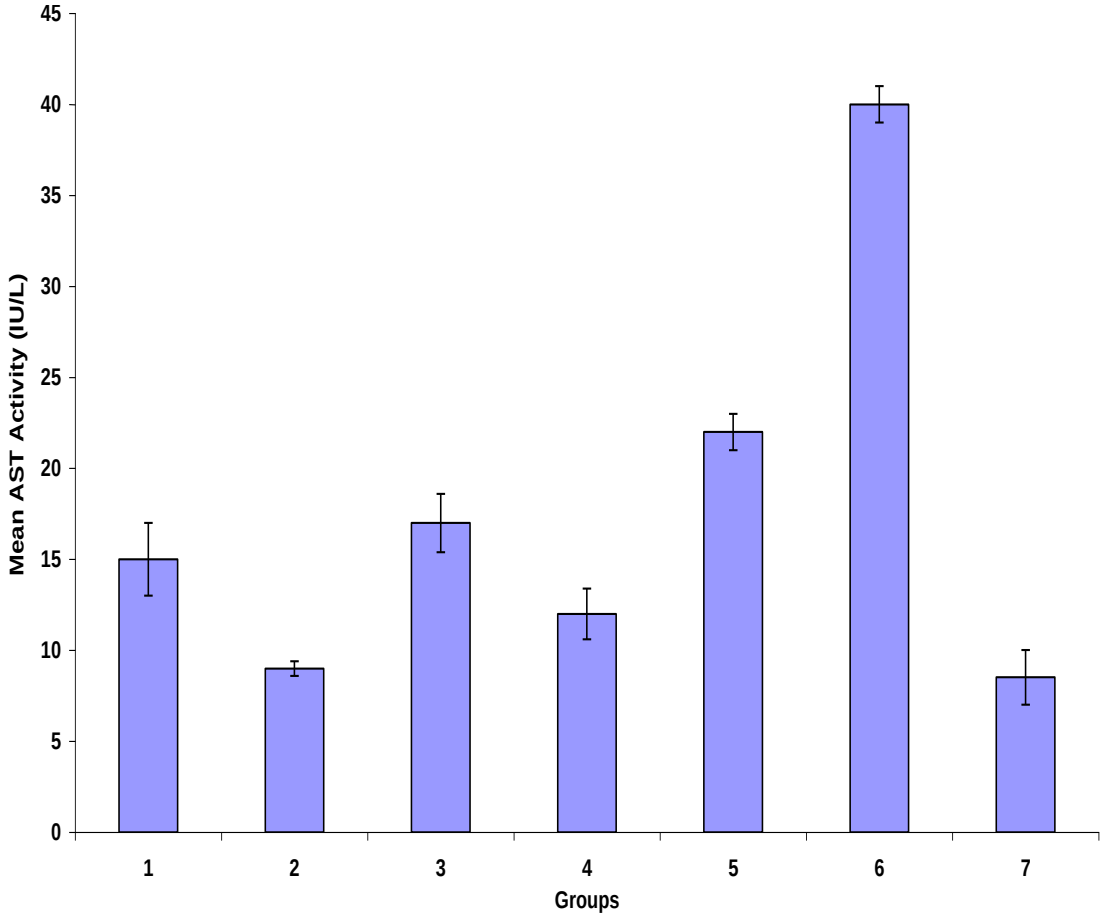

Figure 2. Effects of varying doses of the combined plant extracts on aspartate aminotransferase (AST) activity. Group 1= *A. occidentale* + *E. globulus* (100 mg/kg b.w) Group 2= *A. occidentale* + *E. globulus* (250 mg/kg b.w). Group 3 = *P. guajava* + *X. aethiopica* (100 mg/kg b.w). Group 4 = *P.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

doses of the combined extracts resulted in significant (p<0.05) decrease in ALT 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 weight)] significantly (p<0.05) and dose-dependently reduced the activity of ALT when compared with the value obtained for the diabetic untreated group. However, the 250 mg/kg body weight of *P. guajava* + *X. aethiopica* caused the greatest reduction in the activity of ALT.

Effects of varying doses of the combined plant extracts on aspartate aminotransferase (AST) activity

The AST activities of groups 1, 2, 3, 4, 5 and 7 were significantly (p<0.05) and dose-dependently lower than that of the diabetic untreated group (group 6). There were no significant (p>0.05) differences between the AST activities of groups 2 and 4 and that of the group 7 [non diabetic control group (5 ml/kg body weight of DMSO)] as shown in Figure 2.

Effects of varying doses of the combined plant extracts on alkaline phosphatase (ALP) activity

As shown in Figure 3, the diabetic untreated group had the highest ALP activity but administration of the different doses of the combined extracts caused significant (p<0.05) decrease in ALP activity. The 100 and 250 mg/kg body weight of each of *A. occidentale* + *E. globulus* and *P. guajava* + *X. aethiopica* in a similar man-ner as the standard anti-diabetic drug [glibenclamide (5 mg/kg body weight)] significantly (p<0.05) and dose-relatedly led to a drop in the activity of ALP when com-pared with the value obtained for the diabetic untreated group. The 250 mg/kg body weight of *P. guajava* + *X. aethiopica* however, depressed the activity of ALP best.

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

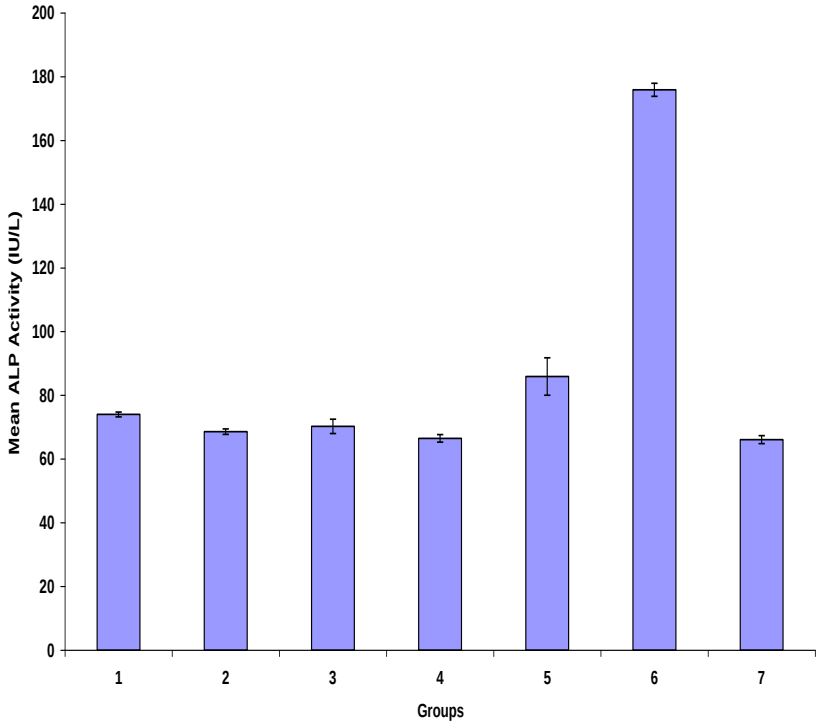

Figure 3. Effects of varying doses of the combined plant extracts on alkaline phosphatase (ALP) activity. Group 1= *A. occidentale* + *E. globulus* (100 mg/kg b.w) Group 2 = *A. occidentale* + *E. globulus* (250 mg/kg b.w). Group 3= *P. guajava* + *X.. aethiopica* (100 mg/kg b.w). Group 4 = *P. guajava*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

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* have low toxicity.

The 250 mg/kg body weight of the combined extract (*A. occidentale* + *E. globulus*) exerted better effects in the treated rats than all the doses of *P. guajava* + *X. aethiopica* considering all the studied parameters. The remarkable reductions observed in ALT and AST activities could be said to have been caused by the hepatocellular and cardiac protection offered by the combined extracts. Hepatic and cardiac tissues release aspartate and alanine aminotransferases and therefore, the elevation of plasma concentrations of these enzymes are indicators of hepatic and cardiac damage as in the case of complications in diabetes mellitus (Crook, 2006). The observation is in support of the report of Ogbonnia et al. (2010) who studied the effect of a poly-herbal formulation on liver function enzymes in diabetic rats. It was noted that the administration of the poly-herbal formulation (which has *X. aethiopica* as one of its component) to rats led to a pronounced decrease in ALT and AST activities in the treated rats. The implication of this is that the combined extracts did not produce harmful effects on both the cardiac or hepatic tissues of the treated rats while in the diabetic untreated group, there were notable elevations in the activities of these two enzymes, an indication of hepatic and cardiac tissue damage.

The notable reduction in serum ALP activity recorded is suggestive of cellular membrane/hepatocellular membrane protective effects of the combined extracts. ALP functions as a biochemical marker enzyme for maintaining membrane integrity. Increase in its plasma activity indicates peroxidation of cell membrane which occurs during diabetes mellitus (Akanji et al., 1993). Uboh et al. (2010) showed that the aqueous extract of *P. guajava* confers hepatocellular protection in rats. The hepatocellular protection evidenced in the present study might be due to the presence of flavonoids in the plant extracts. Flavonoids have been reported to possess antioxidant activity (Middleton, 1996) and thus, are capable of protecting cell membranes from peroxidative actions of free radicals.

In conclusion, this study shows that the combined chloroform extracts of the leaves of *A. occidentale, E. globulus, P. guajava* and fruits of *X. aethiopica* as employed in the study had better decreasing effects on the activities of liver marker enzymes than glibenclamide (a standard anti-diabetic drug) and therefore, may be adopted for the management and/or amelioration of diabetes mellitus and its accompanying complications.

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