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Full Length Research Paper

Effects of some anti-diabetic plants on the hepatic marker enzymes of diabetic rats

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This study was embarked upon in order to evaluate the effects of the chloroform extracts of the leaves of *Psidium guajava*, *Anacardium occidentale* and *Eucalyptus globulus* and fruits of *Xylopia aethiopica* on hepatic marker enzymes of diabetic rats. The degree of hepatic damage caused by diabetes mellitus and the effects of the extracts were assessed using standard methods for assaying the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). All the extracts significantly ($p<0.05$) decreased the activities of ALT, AST and ALP with that of the *P. guajava* being the most protective. In addition, the *P. guajava* extract exerted more hepatoprotection than glibenclamide in terms of the AST and ALP activities. In conclusion, the chloroform extracts of the leaves of *A. occidentale*, *E. globulus* and *P. guajava* as well as the fruits of *X. aethiopica* exhibited remarkable protective effects on alloxan-induced acute liver damage and thus, may be used for treatment of some liver-associated disorders.

Key word: Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), *Psidium guajava*, *Anacardium occidentale*, *Eucalyptus globulus*, *Xylopia aethiopica*, chloroform extracts, diabetes mellitus.

INTRODUCTION

The liver is a vital organ of the body which plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions such as metabolism, secretion and storage. It has a paramount importance in the maintenance and regulation of the homeostasis of the body. It can also detoxify xenobiotics and antibiotics (Ahsan et al., 2009). The liver is capable of utilizing glucose as fuel, storing it in the form of animal starch (glycogen) and synthesizing glucose from non-carbohydrate precursors.

By so doing, it plays a significant role in the regulation of carbohydrate meta-bolism and hence, assisting in the maintenance of normal blood glucose concentrations in both fasting and post-prandial states. Any hepatic damage by way of diabetes mellitus or any other form of metabolic disorder, leads to the distortion of these metabolic functions. Unfortunately, the conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes have serious side effects on the other organs. Herbal drugs or their extracts are prescribed for treatment of liver diseases widely, though their biological active compounds are unknown (Latha and Reddy, 2012).

Insulin dysfunction or lack of it makes the liver undergo glycogenolysis thereby increasing hepatic glucose production. During diabetes mellitus, the unstored fatty acids, abnormal storage of glycerides, together with increased lipolysis in insulin sensitive tissues, such as the liver, results in excess free fatty acids. This has a direct toxic effect on the hepatocytes and in high concentration, culminates in disruption of cell membrane of hepatocytes, leading to the leakage of the hepatic enzymes (Neuschwander-Tetri and Caldweel, 2003). Therefore, some herbal alternatives have proven to be effective in

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providing symptomatic relief and help prevent secondary complications associated with the diseases.

Some plants have also been shown to assist in regenerating beta cells and overcoming insulin resistance (Yasir et al., 2012). This study is directed at investigating the effects of the chloroform extracts of the leaves of *Psidium guajava* (Myrtaceae), *Anacardium occidentale* (Anacardiaceae) and *Eucalyptus globulus* (Myrtaceae) and fruits of *Xylopia aethiopica* (Annonaceae) on hepatic 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 crude extract

The leaves of *A. occidentale*, *E. globulus*, *P. guajava* and 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 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). The doses of each extracts administered was estimated by the methods 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

Fifty (50) male Wistar albino rats of weight (180 to 230 g) and 128 male mice of weight (30 to 40 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 the 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 44 rats (grouped into 11) 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_{50})

Acute oral toxicity study of each plant extract 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

Effects of varying doses of the different plant extracts on ALT activity

Figure 1 shows that upon the administration of varying doses of the different plant extracts to diabetic rats, there was significant ($p<0.05$) reductions in ALT activity. *X. aethiopica* (100 and 250 mg/kg body weight) had the greatest reduction in ALT activity in a dose dependent manner when compared in a descending order to those of *P. guajava* (100 and 250 mg/kg body weight), *A. occidentale* (100 and 250 mg/kg body weight) and *E. globulus* (100 and 250 mg/kg body weight). These reductions in ALT activity caused by the extracts were significantly ($p<0.05$) different from that of the glibenclamide (5 mg/kg body weight), except those of the *P. guajava* (250 mg/kg body weight) and *X. aethiopica* (100 mg/kg body weight).

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

Figure 2 shows that *P. guajava* (100 and 250 mg/kg body weight) significantly ($p<0.05$) decreased AST activity more than as recorded for the groups administered *E. globulus* (100 and 250 mg/kg body weight), *A. occidentale* (100 and 250 mg/kg body weight) and *X. aethiopica* (100 mg/kg body weight). These decreases were in dose dependent manners with the 250 mg/kg body weight of *P. guajava* extract having the greatest reduction ability. The reduction in AST activity caused by the glibenclamide (5 mg/kg body weight) was not significantly ($p>0.05$) different from those of *A. occidentale* (250 mg/kg body weight), *E. globulus* (100 mg/kg body weight) and *X. aethiopica* (100 and 250 mg/kg body weight).

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

As observed in Figure 3, *E. globulus* extract (100 and 250

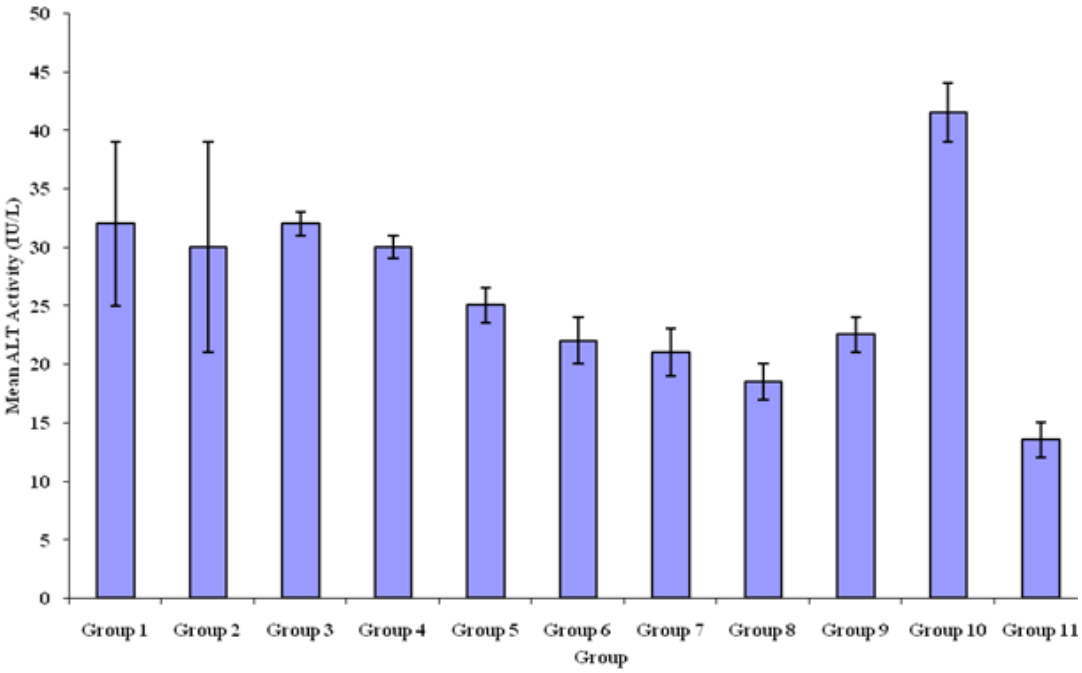

Figure 1. Effect of varying doses of different plant extracts on alanine aminotransferase (ALT) activity in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group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

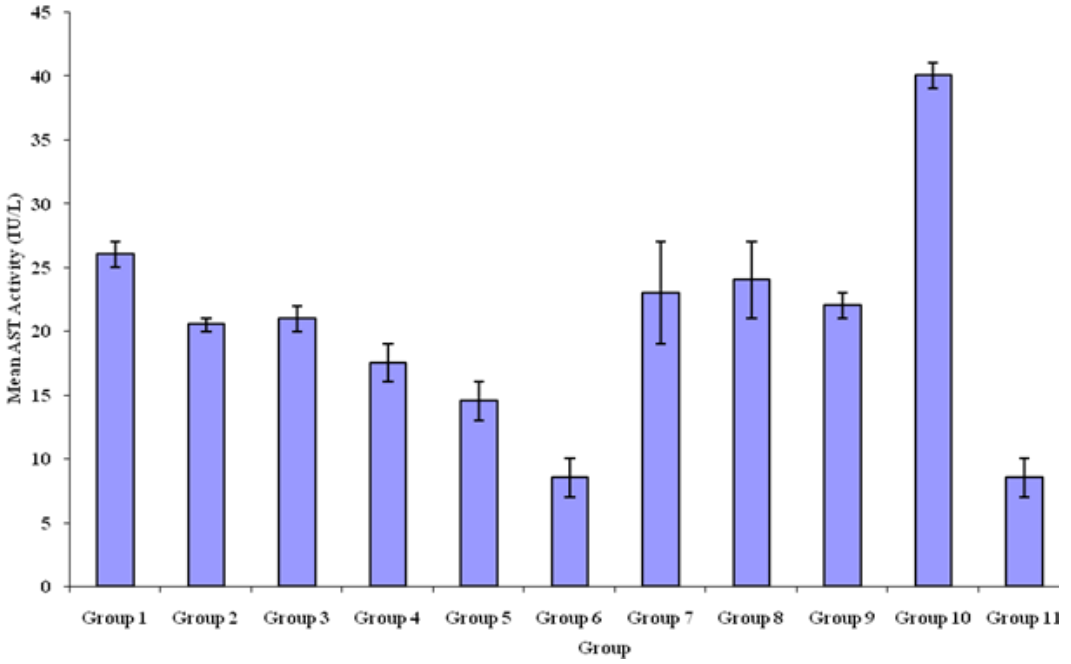

Figure 2. Effect of varying doses of different plant extracts on aspartate aminotransferase (ASP) activity in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg);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

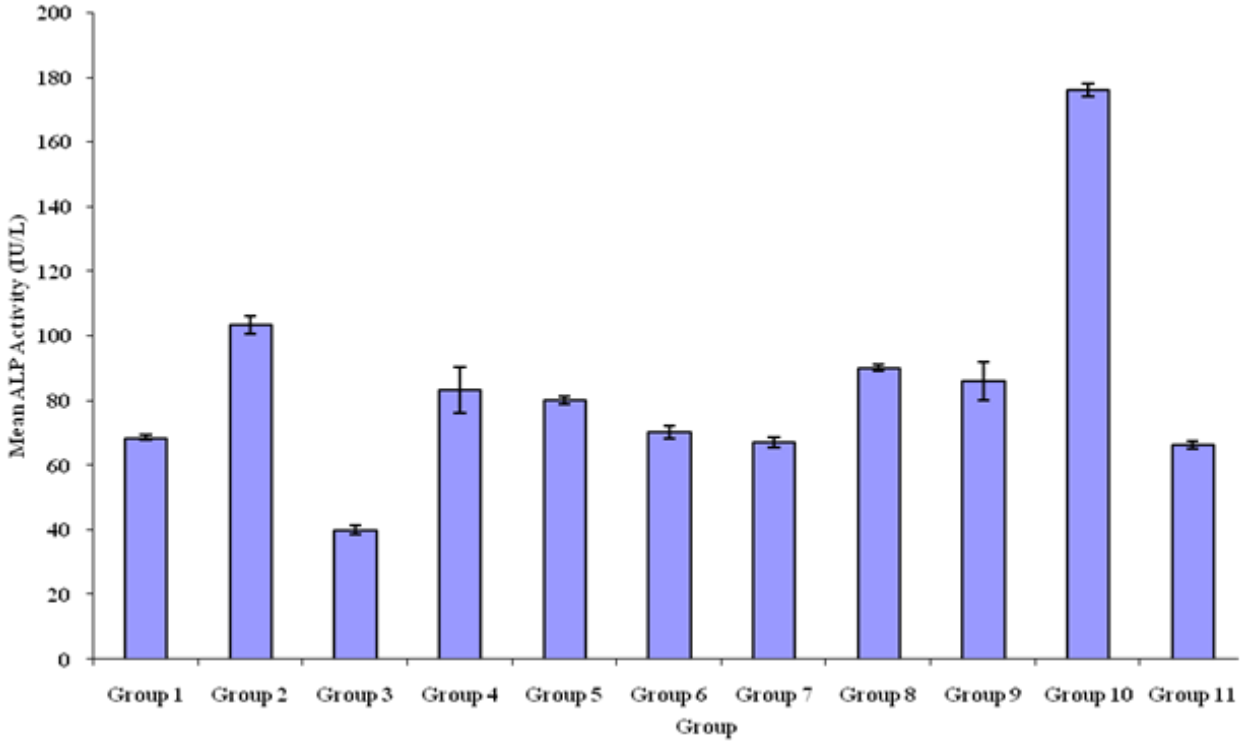

Figure 3. Effect of varying doses of different plant extracts on alkaline phosphate (ALP) activity in rats. Group 1 = *Anacardium occidentale* (100 mg/kg); Group 2 = *Anacardium occidentale* (250 mg/kg); Group 3 = *Eucalyptus globulus* (100 mg/kg); Group 4 = *Eucalyptus globulus* (250 mg/kg); Group 5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

mg/kg body weight) significantly ($p<0.05$) reduced ALP activity when compared to the reductions caused by *P. guajava* (100 and 250 mg/kg body weight). However, the decrease in ALP activity caused by glibenclamide (5 mg/kg body weight) was significantly ($p<0.05$) different from those of *A. occidentale* (100 mg/kg body weight), *E. globulus* (100 mg/kg body weight), *P. guajava* (250 mg/kg body weight) and *X. aethiopica* (100 mg/kg body weight). All the results show that the diabetic untreated groups had significant increases in the activities of all the enzymes assayed when compared to every other test group.

DISCUSSION

The effects of the chloroform extracts of the leaves of *A. occidentale*, *E. globulus* and *P. guajava* and fruits of *X. aethiopica* on ALT, AST and ALP in diabetic rats were investigated in the present study. 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 reductions observed in ALT and AST activities could be said to have been caused by the hepatocellular and cardiac protection offered by these extracts. This is further confirmed by 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 extract did not produce harmful effects on the 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 reduction in serum ALP activity recorded is suggestive of cellular membrane/hepatocellular membrane protective effects of the plant 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, the present study reveals that the chloroform extracts of the leaves of *A. occidentale*, *E. globulus* and *P. guajava* as well as the fruits of *X. aethiopica* showed potent protection on alloxan-induced acute liver toxicity in diabetic rats.

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