



Potentials of *Pterocarpus erinaceus* Poir: Aqueous Stem Bark Extract to Prevent Development of cardiomyopathy in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cardiomyopathy is one of the significant complications of diabetes mellitus. Hyperglycemia plays a central role in the pathogenesis of diabetic cardiomyopathy. The aqueous stem bark extract of *Pterocarpus erinaceus* was investigated for its phytochemical constituents, its effects on blood glucose level, lipid profile, antioxidants markers, and cardiac markers for heart tissue damage in alloxan induced diabetic wistar albino rats. Thirty five (35) rats were randomly divided into six groups. The animals in groups 2-6 were induced with a single dose of 150 mg/kg body weight of alloxan intraperitoneally. They were confirmed hyperglycemic after 72 hours of induction. Group 3 rats were treated with vitamin C as standard drug while group 4-6 were treated with *P. erinaceus* extract (100, 200 and 400 mg/kg body w. t) for 14 days. The results shows that *P. erinaceus* aqueous stem bark extract contains flavonoids as the most abundant constituent with concentration

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of (452.67±7.37 mg/100g). The aqueous stem bark extract given at dose of 100, 200 and 400 mg/kg decrease blood glucose concentration of rats by 44.26%, 47.16% and 74.41% respectively at 14th day of treatment. The rats treated with the plant extract shows significant decrease ($p < 0.05$) in triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) levels as well as significant increase ($p < 0.05$) in the levels of total protein (TP) and high-density lipoprotein (HDL) compared to the diabetic untreated rats. Antioxidant markers such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione transferase (GST) significantly increased ($p < 0.05$) in rats treated with the extract, while malondialdehyde (MDA) level was significantly reduced ($p < 0.05$) compared to the diabetic untreated rats. Serum cardiac markers for heart tissue damage such as cardiac Troponin I (cTnI), aspartate transferase (AST) and creatine kinase-myocardial band (CK-MB) significantly increased ($p < 0.05$) in untreated diabetic rats compared to the diabetic rats treated with the extracts at all doses. Aqueous stem bark extract exhibited antihyperglycemic and antihyperlipidemic activities and mitigated damage to the heart from the alloxan-induced myocardial toxicity associated with type-1 diabetes.

Keywords: *Pterocarpus erinaceus*; diabetic; hyperglycemia; cardiomyopathy; antioxidants.

1. BACKGROUND

Heart disease is rampant in the under developed and developed world, and the epidemic is spreading rapidly around the globe [1]. There are numerous events that contribute to the rise in heart diseases, but the increasing prevalence of diabetes is a marked contributor. Diabetes is a serious metabolic disorder and several of medicinal plants are used in traditional medicine to manage diabetes [2]. Diabetes mellitus (DM) is a persistent metabolic disorder associated with carbohydrate, lipid, and protein metabolisms that contribute to several kinds of complications, including cardiomyopathy (CM) [3]. CM is a disease of the heart muscle that makes it difficult for the heart to pump blood adequately to the rest of the body. CM can lead to heart failure. Diabetic cardiomyopathy (DCM) is defined as the disease process which affects the myocardium in diabetic patients causing myocardial dilatation and hypertrophy, as well as a decrease in the systolic and diastolic function of the left ventricle [4]. DCM is one of the significant complications of diabetes mellitus. It is the most imperative basis of death in 65% of patients with diabetes [3]. Hyperglycemia plays a central role in the pathogenesis of diabetic cardiomyopathy. Cardiac injury may be due to chronic hyperglycemia resulting from the direct and indirect effects of glucose on cardiomyocytes, cardiac fibroblasts, and endothelial cells [4]. Chronic hyperglycemia stimulate the overproduction of reactive oxygen species (ROS) inducing apoptosis and activates poly (ADP-ribose) polymerase-1 (PARP) enzyme that mediates direct ribosylation and inhibition of glyceraldehyde phosphate dehydrogenase (GAPDH) and diverts glucose from the glycolytic

pathway toward alternative biochemical series such as increases in advanced glycation end products (AGEs), and the activation of the hexosamine pathway, the polyol pathway, and protein kinase C that eventually lead to hyperglycemia-induced cellular injury [5,6]. These and other conditions like it are diseased conditions that are traditionally controlled or treated with medicinal herbs such as *Ageratum conyzoides* and *Pterocarpus erinaceus*.

Pterocarpus erinaceus (family: *Leguminosae papilionoideae*) is a deciduous legume tree of African savannas and dry forest. It is commonly known as Madobia or Maijini (Hausa) and African rosewood (English). Decoction from the leaves are used in abortifacient mixtures and as a febrifuge [7]. The bark is used for treatment of scalp infections due to ringworm, for chronic ulcers, blennorrhagia and is gargled for tooth and mouth troubles [7]. The trunk and root bark are used in malnutrition, debility, pregnancy and anaemia [8,9]. The aim of this study is to determine potential of *Pterocarpus erinaceus* aqueous stem bark extract to prevent the development of cardiomyopathy in alloxan induced diabetic albino rats.

2. MATERIALS

2.1 Sample Collection and Identification

The stem bark used in this study is that of *Pterocarpus erinaceus* (Madobia). It was obtained from Gudi, Akwanga Local Government Area, Nasarawa State, Nigeria. The plant was identified and authenticated by International Centre for Ethno Medicine and Drug development. The voucher specimen was deposited in the herbarium with a voucher number (Intercedd/364980).



Fig. 1. *Pterocarpus erinaceus* (Madobia) plant

2.2 Sourcing of Experimental Animals and Housing

Thirty five (35) male adult albino rats weighing between 104-226g were purchased from National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria. The animals were acclimatized for 1 week before induction of diabetes. They were housed under a 12 h/12 h light/dark cycle at $26 \pm 2^\circ\text{C}$ temperature and fed with pelletized food and water.

3. METHODOLOGY

3.1 Sample Preparation and Extraction

Fresh *Pterocarpus erinaceus* stem bark was washed with clean water and dried under shade. Dried sample was pulverized to powder using mortar and pestle. Five hundred gram (500g) of the pulverized stem bark was used for extracts production. Maceration method was used as described by Ewansiha *et al.* (2012). Five hundred grams (500 g) of the pulverized *Pterocarpus erinaceus* stem bark was soaked in 2500 ml of hot water and stirred. After two days, the mixture was filtered with muslin cloth (folded twice) followed by Whatmann filter paper. The filtrate was concentrated, then cooled in a glass bottle and stored in the refrigerator as crude extract.

3.2 Induction of Diabetes

All rats were induced with diabetes using alloxan except those that were used as normal control after overnight fast. Freshly prepared normal saline solution of alloxan was injected intraperitoneally to the rats at a single dose of 150 mg/kg body weight of animal. After 72 hours of induction of diabetes (3 days), blood was taken from each rat to estimate blood glucose

levels to confirm establishment of diabetes. Rats with fasting blood glucose concentration of ≥ 200 mg/dl were considered diabetic and used in the study as reported by [10].

3.3 Experimental Design and Treatment

The diabetic rats were randomized into five (5) groups with six (6) rats each; group 2 and 3 served as diabetic control and standard control respectively, while group 4, 5 and 6 received graded doses of the extract by gavages; and group 1 received normal saline. The hyperglycemia was sustained and stabilized for 3 days, and the treatment continued for two weeks (14 days). The experimental design and treatment are summarized below:

- Group 1: Normal Control + Normal saline
- Group 2: Diabetic control
- Group 3: Diabetic + Vitamin C (25 mg/kg)
- Group 4: Diabetic + Extract (100mg/kg)
- Group 5: Diabetic + Extract (200mg/kg)
- Group 6: Diabetic + Extract (400mg/kg)

3.4 Fasting Blood Sugar

The fasting blood sugar was recorded on 0, 7th and 14th day of treatment.

3.5 Animal Sacrifice and Sample Collection

After two weeks (14 days) of treatments, the animals were anesthetized with diethyl ether. The blood was collected through the retro orbital plexus into sample bottles devoid of the anticoagulant. The samples were centrifuged at 4000 rpm for 5 minutes to obtain the sera for determination of lipid profile and cardiac markers. Each animal's cardiac tissue was excised and homogenized to obtain heart homogenate for determination of *in vivo* antioxidant activity.

3.6 Determination of Phytochemical Composition of *P. erinaceus* Aqueous Stem Bark Extract

Quantitative determination of phytochemicals composition of aqueous stem bark extracts of *Pterocarpus erinaceus* was carried out using standard phytochemical screening procedures

3.7 Determination of *In vivo* antioxidant Parameters and Lipid Peroxidation in Homogenate of Heart Tissues

The following biomarkers were determine to assess oxidative stress: superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione -S-transferase (GST) and catalase activity (CAT) were measured with highly specific enzyme-linked immunosorbent (ELISA) kit according to the procedure provided in the kit manuals. Malondialdehyde (MDA, an index of lipid peroxidation was determined using the method reported by Buege and Aust, (1978) [11].

3.8 Assay of Lipid Profile

Total cholesterol (TC), total triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-Chol) levels were determined by using previously modified enzymatic procedures. Low-density lipoprotein-cholesterol (LDL-Chol) levels was calculated using the Friedewald equation [12].

3.9 Cardiac Biomarkers

Cardiac Troponin I (cTnI) concentration, Creatine Kinase-myocardial band (CK-MB) activity and

Aspartate aminotransferase (AST) activity were measured with highly specific enzyme-linked immunosorbent (ELISA) kit according to the procedure provided in the kit manuals.

3.10 Statistical Analysis

Statistical package for social sciences (SPSS) software version 23 was used for statistical analysis. All result were expressed as mean \pm SD. Mean values in all groups were compared using one-way analysis of variance (ANOVA) with n=3. And Duncan's post hoc test were employed to test the significance of difference across the groups and between the groups; and p < 0.05 was considered statistically significant. The decreases in percentage of glucose level in experimental animal was calculated by formula given below according to [13].

$$\% \text{ Decrease in blood glucose level} = \frac{(\text{Before treatment} - \text{After treatment})}{\text{Before treatment}} \times 100$$

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Quantitative analysis

The results of quantitative phytochemical composition of aqueous extract of *Pterocarpus erinaceus* stem bark are presented in Fig. 2. Flavonoids has the highest concentration (452.67 \pm 7.37) amongst all the phytochemicals present.

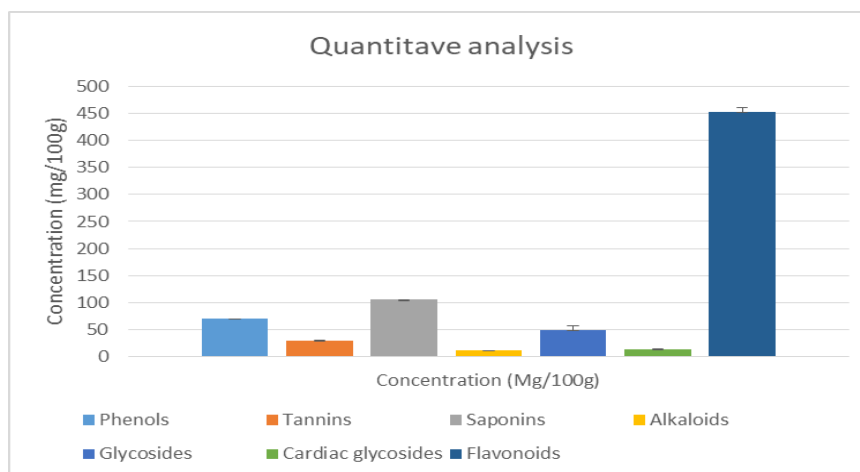


Fig. 2. Quantitative phytochemical composition of aqueous extracts of *Pterocarpus erinaceus* stem bark

Results are expressed as Mean \pm SD, n=3

4.1.2 The effect of oral administration of aqueous stem bark extract of *Pterocarpus erinaceus* (PE) on blood glucose level of alloxan induced diabetic wistar albino rats

Fig. 3 shows the hypoglycemic effect of aqueous stem bark extract of *P. erinaceus* on alloxan induced diabetic albino rats. The aqueous stem bark extracts of PE at dose of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight brought about 44.26% (213.20 ± 9.90 to 118.80 ± 14.40), 47.26% (216.30 ± 1.00 to 114.30 ± 2.70) and 74.41% (333.00 ± 11.20 to 85.20 ± 11.71) reduction in blood glucose levels respectively when comparing between day 0 and the day 14 of treatment. While the standard drug vitamin C brought about 51.09% (248.40 ± 3.60 to 121.50 ± 4.50) reduction in blood glucose level when comparing between the day 0 and day 14. At the 14th day, diabetic untreated group showed a significant increase ($p < 0.05$) in blood glucose level when compared with the day 0 (206.13 ± 0.91 to 304.20 ± 2.00).

4.1.3 The effect of oral administration of aqueous stem bark extract of *P. erinaceus* on Lipid Profile in alloxan induced diabetic rats.

The results of the effect of oral administration of aqueous extract of *P. erinaceus* stem bark in Fig. 4 showed significant reduction ($p < 0.05$) in Total Cholesterol (T. Chol), Triglycerides (TG) and Low-density lipoprotein (LDL) values of rats

treated with aqueous stem bark extracts of PE at the doses of 100, 200 and 400 mg/kg body weight respectively when compared to diabetic untreated rats. Also, groups treated with PE extracts at doses of 100, 200 and 400 mg/kg body weight showed significant increase ($p < 0.05$) in HDL and TP concentration when compared to the diabetic untreated group.

4.1.4 The effect of oral administration of aqueous stem bark extract of PE on antioxidants and lipid peroxidation marker (MDA) in alloxan induced diabetic rats.

In Fig. 5, the study showed that diabetic untreated group significantly decrease ($p < 0.05$) in CAT (746.47 ± 52.16 U/mg), SOD (208.34 ± 1.01 U/mg), and GPx (156.31 ± 6.34 U/mg) activities when compared to normal control rats. Diabetic rats treated with *P. erinaceus* and vitamin C significantly increased ($p < 0.05$) in cardiac CAT, SOD and GPx activities at all doses when compared to the diabetic untreated rats. Also, a significant increase ($p < 0.05$) in MDA content (5.56 ± 0.49) was observed in the diabetic untreated group when compared to the normal animals while diabetic rats treated with *P. erinaceus* extracts had significantly reduced the MDA level at all doses compared to the diabetic untreated group. GSH and GST significantly increase ($p < 0.05$) in diabetic rats treated with *P. erinaceus* and vitamin C compared to the diabetic untreated rats.

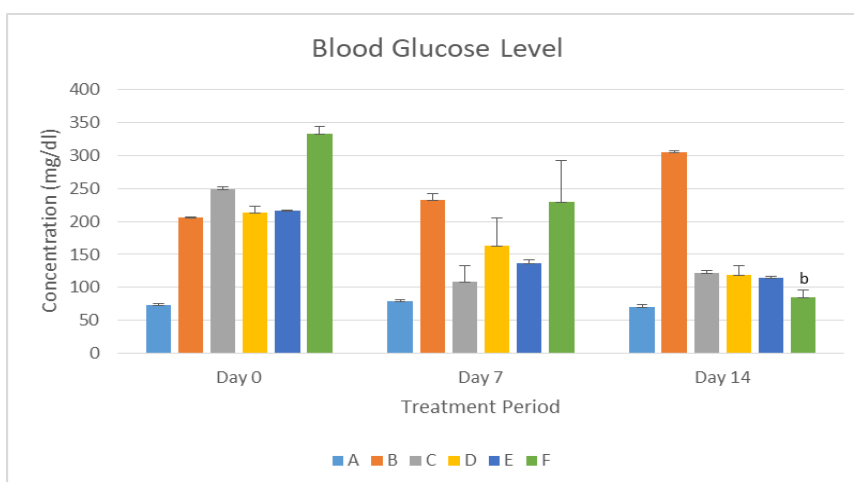


Fig. 3. The effect of oral administration of aqueous stem bark extract of *P. erinaceus* on blood glucose level of alloxan induced diabetic wistar albino rats

Values are expressed as Mean \pm SD, n=3

^b: There is significant difference with $p < 0.05$ when comparing between day 0 and day 14 of treatment
 A=Normal control; B=Diabetic control; C= Diabetic + 25 mg/kg Vitamin C; D=Diabetic + 100 mg/kg PE;
 E=Diabetic + 200 mg/kg PE; F= Diabetic + 400 mg/kg PE; PE=*Pterocarpus erinaceus*, b. wt= body weight.

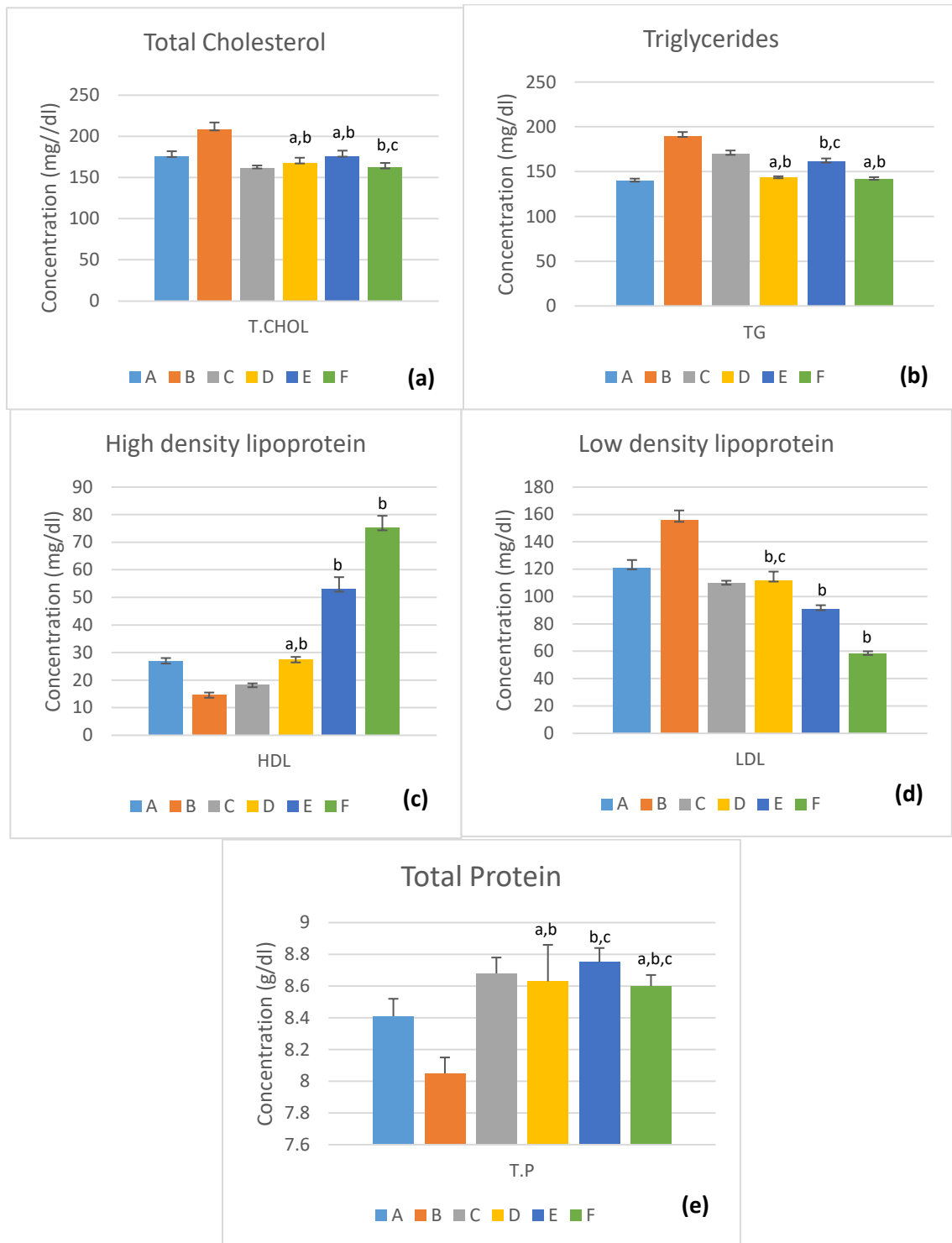


Fig. 4. The effect of oral administration of aqueous stem bark extract of *P. erinaceus* on Lipid Profile in alloxan induced diabetic rats

Values are expressed as Mean \pm SD, n=3

^a: There is no significant difference when compared with the Normal control at $p > 0.05$

^b: There is significant difference when compared with the diabetic control at $p < 0.05$

^c: There is no significant difference when compared with the Vitamin C at $p > 0.05$

A=Normal control; B=Diabetic control; C= Diabetic + 25 mg/kg Vitamin C; D=Diabetic + 100 mg/kg PE; E=Diabetic + 200 mg/kg PE; F= Diabetic + 400 mg/kg PE; PE=*Pterocarpus erinaceus*, b. wt= body weight

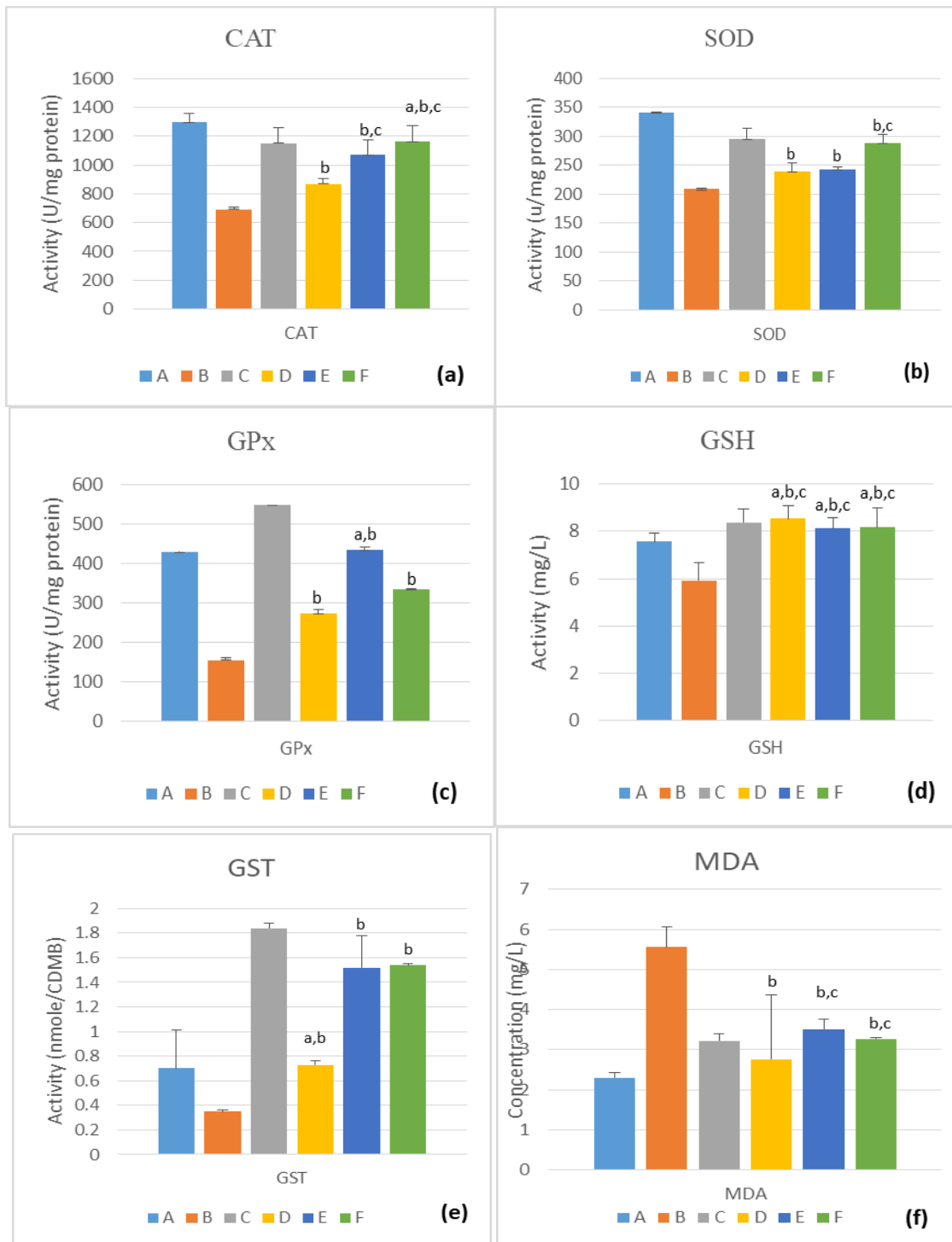


Fig. 5. The effect of oral administration of aqueous stem bark extract of *P. erinaceus* on antioxidants (GSH and GST) and lipid peroxidation marker (MDA) in alloxan induced diabetic rats

Values are expressed as Mean \pm SD, n=3

^a: There is no significant difference when compared with the Normal control at $p>0.05$

^b: There is significant difference when compared with the diabetic control at $p<0.05$

^c: There is no significant difference when compared with the Vitamin C at $p>0.05$

A=Normal control; B=Diabetic control; C= Diabetic + 25 mg/kg Vitamin C; D=Diabetic + 100 mg/kg PE; E=Diabetic + 200 mg/kg PE; F= Diabetic + 400 mg/kg PE; PE=*Pterocarpus erinaceus*, b. wt= body weight

4.1.5 The effect of oral administration of aqueous stem bark extract of PE on serum cardiac biomarkers (cTnI, CK-MB and AST) of alloxan-induced diabetic rats

From Fig. 6, the mean concentration of cardiac troponin I (cTnI) in serum of diabetic control rats showed a significantly ($p < 0.05$) increased value (6.54 ± 0.61 ng/ml) compared to the normal control group which showed lower value (0.61 ± 0.07 ng/ml) for the cTnI concentration. The groups treated with the PE extract at various doses showed a significant reduction ($p < 0.05$) in serum cTnI concentration compared to the diabetic control group. The level of serum Aspartate transferase (AST) level as marker of heart damage was significantly increased ($p <$

0.05) in diabetic control rats with value of 32.11 ± 1.00 U/l compared to the normal control group which showed value of 27.43 ± 0.56 U/l. Treatment with PE extract at doses of 100, 200 and 400 mg/kg b. wt showed significant reduction ($p < 0.05$) with AST values of 22.90 ± 2.37 , 23.16 ± 1.90 U/l and 19.83 ± 3.90 U/l respectively when compared to the diabetic control group. Diabetic untreated rats showed a significant increase ($p < 0.05$) in the serum creatine kinase (CK-MB) activity level as compared to the normal control rats. Groups treated with PE extract significantly decrease ($p < 0.05$) the serum CK-MB activity level when compared to diabetic untreated group, whereas, these plant treated groups showed no significant difference when compared to the normal control group.

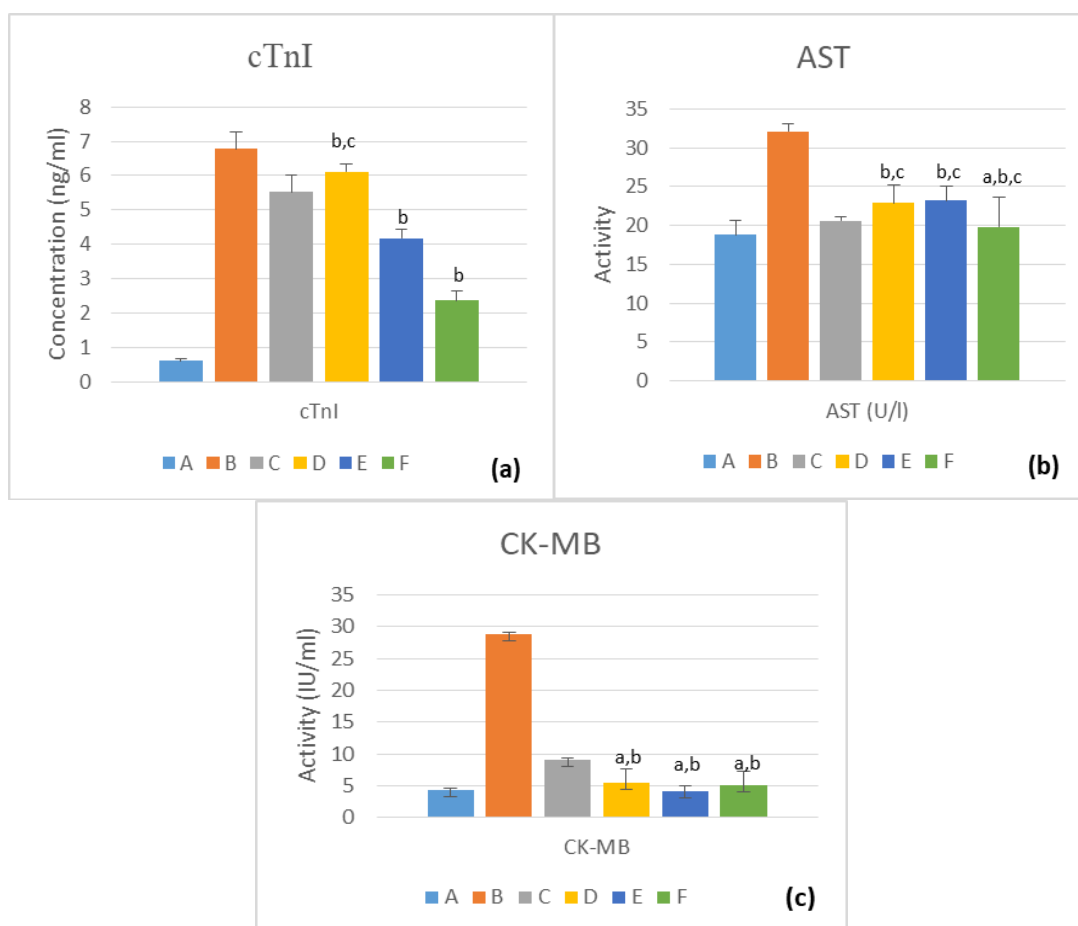


Fig. 6. The effect of oral administration of aqueous stem bark extract of PE on Serum cTnI, CK-MB and AST of alloxan induced diabetic wistar albino rats

Values are expressed as Mean \pm SD, n=3

^a: There is no significant difference when compared with the Normal control at $p > 0.05$

^b: There is significant difference when compared with the diabetic control at $p < 0.05$

^c: There is no significant difference when compared with the Vitamin C at $p > 0.05$

A=Normal control; B=Diabetic control; C= Diabetic + 25 mg/kg Vitamin C; D=Diabetic + 100 mg/kg PE; E=Diabetic + 200 mg/kg PE; F= Diabetic + 400 mg/kg PE; PE=Pterocarpus erinaceus, b. wt= body weight

4.2 Discussion

Phytochemical screening of *P. erinaceus* aqueous stem bark showed the presence of alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, saponins, and tannins (Nzute et al., 2023, potential of *Pterocarpus erinaceus* aqueous stem bark extract to prevent oxidative kidney damage in diabetic rats; unpublished). These phytochemicals were also reported to be present in *P. erinaceus* stem bark [7,14,15]. The quantitative phytochemical analyses revealed flavonoids as the most abundant constituent with concentration of (452.67±7.37 mg/100g), followed by saponins (105.18±0.70 mg/100g) and phenols (69.72±0.58 mg/100g). Phytochemicals such as saponins, flavonoids and alkaloids have been shown to have anti-inflammatory effects [16]. Flavonoids are free radical scavengers, they prevent oxidative damage and have strong anticancer activity [17], and they also function to protect against allergies, inflammation, ulcer and tumor [18]. The presence of flavonoids and tannins have been reported to be antioxidants used to neutralize highly unstable and extremely reactive molecules like free radicals that attack the cells of human body [19,20].

After 14 days of administration of *P. erinaceus* aqueous stem bark extract, blood glucose concentration decreased by 44.26%, 47.16% and 74.41% at doses of 100, 200 and 400 mg/kg b. wt respectively in comparison with day 0 of treatment. The decreased observed could be as a signs of regeneration of β cells, potentiating insulin secretion from surviving β cells of the islets of langerhans following consumption of the stem bark extracts. *P. erinaceus* stem bark may have some chemical components that exerts regenerative effects on β cells, and stimulate these cells to produce more insulin (pancreatotropic action). Induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreases [21]. The decrease in blood glucose level could be as a result of the presence of flavonoids and tannins in the aqueous stem bark extracts.

Significant reduction in serum total protein concentrations in the group induced with alloxan without treatment may suggests the feature of untreated diabetes and advance chronic liver damage. Hypoproteinemia is a feature of advanced chronic liver disease [22]. The significant rise ($p<0.05$) in serum TP and HDL

concentration of groups treated with PE stem bark extract as well as the significant reduction ($p<0.05$) in LDL concentration of groups treated with the extract at doses of 100, 200 and 400 mg/kg when compared with diabetic control group might be showing the potentiality of the plant extract in managing diabetes and preventing tissue damage caused by chemical such as alloxan. Dyslipidemia is a major risk factor for cardiac, cerebral, and renal complications [23].

The body has evolved a complex defence strategy to minimize the damaging effects of various oxidants. Central to this defence are the non-enzymatic and enzymatic antioxidants [24]. These include reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and catalase (CAT) respectively, which act in concert to protect the organism from oxidative damage [25]. GPx, SOD and CAT are the primary free radical scavenging enzymes involved in the first line of cellular defense against oxidative injury, removing O_2^- and H_2O_2 before they can interact to form more reactive hydroxyl radicals [26,27]. This work evaluated the effect of extracts on antioxidants and oxidative stress parameters MDA, GSH, GPx, GST, SOD and CAT on heart tissue which oxidative stress appears to play a crucial role in cardiovascular diseases. Diabetic rats treated with the *P. erinaceus* markedly alleviate the oxidative damage by alloxan induced in rats. It was observed that the administration of *P. erinaceus* upturned the enhanced lipid peroxidation and the consequent decline in the level of enzymatic antioxidant (GPx, SOD, GST and CAT) as well as the activities of non-enzymatic antioxidant (GSH) in the cardiac tissue of diabetic rats.

Concentration of cardiac troponin I (cTnI), creatine kinase- myocardial band (CK-MB) and aspartate transferase (AST) activity were measured as serum biomarkers of myocardial injury. cTnI is considered as one of the most sensitive and commonly used biomarkers, and its release from the cardiomyocytes is proportional to the size and extent of cardiac tissue injury [28]. It has been reported by Robertson et al., (2004); Goyal and Patel, (2011) that the cytotoxic action of alloxan is mediated by the formation of free radicals such as superoxide and catalase radicals, which selectively damages the β cells of the pancreas, which are known to be one of the weakest structures to oxidative stress by

generating excess reactive oxygen species and produces heart lesions that are similar to human diabetic cardiomyopathy [29]. Excessive ROS production that exceeds critical levels can overcome all the heart's antioxidants' protection strategy, causing oxidative stress that damages the biological tissues in the hearts [29]. In the present study, diabetic control group showed a marked increase in cTnI concentration indicating that there is an extensive damage to the cardiac tissues in the presence of alloxan. However, the treatment with PE aqueous stem bark extract showed a marked reduction of the cTnI value suggesting that this plant extract has the ability to reduce cardiac cell injury caused by oxidative stress. Serum CK-MB activity was found to be increased in diabetic untreated rats, this may be due to myocardial dysfunction. Hall, (1991); Hagar, (2002) reported that serum CK-MB activity was found to be increased in cardiac muscular damage and serve as a cardiovascular risk-related marker [3]. The significant decrease in serum CK-MB level observed from this study could suggest the beneficial effects of this plant in reducing cardiovascular risk in diabetes mellitus. Therefore, the potential of *P. erinaceus* aqueous stem bark to prevent the development of cardiomyopathy as observed from the result could be due to its phytochemical composition of flavonoids, saponins, phenols etc.

5. CONCLUSION

The relative abundance of flavonoids as the most abundant phytochemical component of *P. erinaceus*, followed by saponins and phenols have justified the use of *P. erinaceus* in traditional medicine for the treatments of diabetes and other disease conditions. The overall biochemical results of the present study shows that aqueous extract of *P. erinaceus* stem bark had the potential to significantly reverse the increased values of cardiac biomarkers of myocardial injury and oxidative stress markers. All these attenuation effects may be attributed by cardioprotective, and antioxidative, effects of *Pterocarpus erinaceus* stem bark extract.

ETHICAL CLEARANCE

Ethical clearance was received from NSUK animal care and use research ethics committee (NSUK-ACUREC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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