Nigerian Journal of Biochemistry and Molecular Biology 2017; 32(1): 10-22

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.com.ng

NJBMB/013/17

Cardioprotective Activities of *Piper guineense* Seeds on Isoproterenol-treated Rats

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Abstract: This study investigated the effects of *Piper guineense* seed extract against isoproterenol-induced myocardial infarction in Wistar rats with a view to employing the plant in the management of heart related disorders. Thirty adult Wistar rats were randomly divided into 6 groups of 5 rats each, treated with the extract of *P. guineense* (125 and 250 mg/kg body weight [bwt]) and myocardial infarction was induced by intraperitoneal injection of isoproterenol (ISO) at 80 mg/kg bwt. The activities of cardiac biomarkers, enzymatic and non-enzymatic antioxidants, lipid profile, total protein and sugar were evaluated using standard methods. Histological examinations of the heart were done. Administration of ISO (80 mg/kg bwt) caused significant ($p < 0.05$) elevation of the activities of cardiac biomarkers, creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) which were significantly ($p < 0.05$) reduced by pre-treatment with the extract. The lipid profile, total sugar, enzymatic and non-enzymatic antioxidants perturbed by the administration of ISO were restored (except the activity of superoxide dismutase which was increased). Histological examinations of sections of the heart indicated myocardial injury while pre-treated animals showed lesser degree of myocardial damage in ISO treated rats. The study concludes that extract of *P. guineense* seeds protected against ISO-induced myocardial infarction in Wistar rats.

KEYWORDS: Antioxidant enzymes, Cardiac biomarkers, Cardioprotection, Piperaceae, *Piper guineense*

1.0 Introduction

Healthy living is always cardinal to human beings, starting from birth to the end of life. However, with modernization as well as sophistication in life, human health directly or indirectly face challenges from several diseases resulting sometimes in survival and sometimes in surrender to diseases (Upaganlawar *et al.*, 2011). One of these diseases is cardiovascular diseases (CVDs), which remain the principal cause of death in both developed and developing countries (WHO, 2015). It presents as a typical 'heart attack', sudden death, or as a silent infarct (Upaganlawar *et al.*, 2011; WHO, 2015).

CVDs include high blood pressure, coronary heart disease, congestive heart failure, stroke which account for 17.5 million deaths per annum worldwide (WHO, 2015). Globally, myocardial infarction is a major cause of death and disability. The classical symptoms associated with myocardial infarction include acute coronary syndrome, chest pain, shortness of breath, nausea, vomiting, palpitations, sweating, and anxiety (Thygesen *et al.*, 2007).

Isoproterenol (ISO), a sympathomimetic β-adrenergic receptor agonist which causes severe stress to the myocardium resulting in an infarct-like necrosis of heart muscle and produces stimulation that increases its rate and force of contraction (Senthil *et al.*, 2007). It is useful in the treatment and management of atrioventricular block or cardiac arrest. It increases systolic blood pressure slightly, but greatly reduces mean arterial and diastolic blood pressure. ISO-induced myocardial infarction is a widely used experimental model due to its extraordinary technical simplicity, excellent reproducibility, acceptable and low mortality (Senthil *et al.*, 2007; Upaganlawar *et al.*, 2011).

Myocardial infarction induced by ISO has been reported to exhibit many metabolic and morphologic aberrations in the heart tissues of

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experimental animals similar to those observed in human myocardial infarction (Radhika *et al.*, 2013). It is maximal in the sub-endocardial region of the left ventricle and in the interventricular septum (Upaganlawar *et al.*, 2011). In addition, oxidative stress has been implicated as one of the main mechanisms through which ISO exerts its toxic effects. Auto-oxidation of ISO produces quinones, which interact with sulphydryl groups of various proteins leading to the production of superoxide anions and hydrogen peroxide (Upaganlawar *et al.*, 2011). The free radicals generated changes the microsomal permeability and mitochondrial Ca^{2+} uptake. It also decrease ATP production and enhance the formation of highly reactive hydroxyl radicals which cause protein, lipid and DNA damage (Dhalla *et al.*, 2010).

P. guineense commonly known as West African "black pepper" is a climbing perennial plant belonging to the family, Piperaceae. It is found in the tropical regions of Central and Western Africa, and similar to cubeb pepper in terms of flavour. It has a less bitter and fresher, herbaceous flavour. It is a spice whose fruits contain pungent piperine that provides essential oil used in the beverage and pharmaceutical industries (Opara, 2014; Oyemitan *et al.*, 2014). The pungency of the 'pepper' is due to the presence of resins particularly 'chavicine' and yellow alkaloid, piperine, in the fruits that constitutes 5 - 8% of the weight of black pepper (Opara, 2014).

P. guineense has been reported to possess medicinal and health benefits including the treatment and prevention of morning sickness, allergy, running nose and cold (Ekanem *et al.*, 2010; Hassan *et al.*, 2010; Etim *et al.*, 2013; Tankam and Ito, 2013; Opara, 2014). However, the cardioprotective activity of the plant has not been investigated, hence the present study.

2.0 Materials and Methods

2.1 Materials

2.1.1 Reagents and Chemicals

All chemicals used in the study were of analytical grade and were purchased from British Drug House Chemicals Limited, Poole, England. 2, 2'- α-bipyridyl, epinephrine, bovine serum albumin, L-Ascorbic acid, reduced glutathione, heptane and Folin-Ciocalteu's Phenol reagent were obtained from Sigma-Aldrich Laboratories, Switzerland. Isoproterenol (Isoprenaline hydrochloride) was purchased from Adooq Bioscience, USA. Diagnostic kits for the assays of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), total protein (TP), triacylglycerol (TG), total cholesterol (TC) and high density lipoproteins (HDL-C) were obtained from Randox Laboratories Ltd, United Kingdom.

2.1.2 Plant Material

Seeds of *P. guineense* were purchased from a local market in Ile-Ife, Nigeria. Identification and authentication were carried out at Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where specimen copy (IFE 17480) was deposited.

2.1.3 Experimental Animals

Wistar rats (30) of either sex weighing between 200 and 250 g were bought from the Animal House of the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. The animals were housed in polypropylene cages lined with wood shavings, replaced every 24 hours, with 12 hours of light and dark. They were fed with standard rat chow (Ladokun Feeds, Ibadan, Nigeria) and provided with water *ad libitum*. The animals were left to acclimatize for four weeks before the commencement of administration of extracts. All the experimental animals were kept in a clean environment. The experiments were performed according to the Animal Experiment Ethical Rules of University of Ibadan.

2.2 Methods

2.2.1 Preparation of Methanol Extract of P. guineense

The seeds were sun-dried pulverised and defatted with n-hexane (100 mL x 5) using Soxhlet extractor. The methanol seed extract of *P. guineense* was prepared as reported earlier

(Oyedapo and Amos, 1997). Typically, 100 g of defatted powdered seed were extracted in 500 mL of methanol-water mixture (3:1 v/v) for 72 hour at room temperature with intermittent shaking. The suspension was filtered using Whatman No. 1 filter paper. The residue was rinsed with the extracting solvent and re-extracted five more times until the extract became colourless. The filtrates were combined, filtered again and concentrated *in vacuo* to dryness under reduced pressure at 40°C on Vacuum Pump V-700 Rotary Evaporator (Buchi Corporation, Switzerland). The dark brown residue of methanolic extract of *P. guineense* (termed MEPG) was kept under sterile condition in the dessicator at 4°C for further analysis.

2.2.2 Animal Grouping and Treatment

The experimental rats were divided into six groups of five animals each and treated as follows:

- Group I: Rats + distilled water (Control)
- Group II: Rats + ISO (80 mg/kg bwt) (Li *et al.*, 2012; Prabha *et al.*, 2014)
- Group III: Rats + extract (125 mg/kg bwt) + ISO (Omodamiro and Jimoh, 2014)
- Group IV: Rats + extract (250 mg/kg bwt) + ISO
- Group V: Rats + extract (250 mg/kg bwt)
- Group VI: Rats + reference drug (propranolol, 1.8 mg/kg bwt) + ISO

Distilled water, propranolol or extract were administered orally to experimental rats, once daily for 28 consecutive days. The rats (other than groups I and V) were challenged with intraperitoneal dose of isoproterenol at an interval of 24 hours for 2 days to induce experimental cardiotoxicity/myocardial infarction. Animals were sacrificed 24 hours after the last dose of isoproterenol.

2.2.3 Preparation of Plasma and Heart Supernatant

The plasma was prepared as reported by Olagunju *et al* (2000). The blood was collected by cardiac puncture into tubes containing EDTA. The collected blood samples were centrifuged (90-2 Microfield Instrument, Essex, England) at 3000 rpm for 20 minutes. The supernatant (plasma) was carefully removed with Pasteur pipette into sterile vial, kept in the deep freezer and used for the analysis of biochemical parameters.

The heart homogenates (10% w/v) were prepared according to the method reported by Bode and Oyedapo (2011). The hearts were surgically removed and immediately rinsed in normal saline (0.85% w/v NaCl) to remove blood cells. The heart (1 g) was cut into thin slices and homogenized in 10 ml of freshly prepared 100 mM phosphate buffer, pH 6.8. The homogenates were centrifuged at 3000 rpm for 10 minutes. The supernatants were carefully transferred into clean vials and stored frozen for further biochemical assays.

2.2.4 Biochemical Analyses

2.2.4.1 In vivo Antioxidant Enzyme Assay

2.2.4.1.1 Glutathione Peroxidase (GPx)

The assay of GPx activity was carried out according to the method described by Rotruck *et al.* (1973) based on catalytic oxidation of glutathione by hydrogen peroxide (H_2O_2). The GPx activity was determined using the expression:

GPx activity (µmol/mg protein) =

$$\frac{Abs_{412} \times \text{Total Assay Volume} \times DF}{\varepsilon \times \text{Sample Volume}}$$

Where Abs_{412} = Absorbance at 412 nm, DF = dilution factor, $\varepsilon = 6.22 \times 10^3$

2.2.4.1.2 Superoxide Dismutase (SOD) Activity

The assay of hear supernatant superoxide dismutase activity was carried out according to the method of Misra and Fridovich (1972) based on the inhibition of auto-oxidation of epinephrine to adrenochrone at pH 10.2.

$$\text{Inhibition (\%)} = \frac{\text{Increase in Absorbance of Substrate} \times 100}{\text{Increase in Absorbance of Blank}}$$

One unit of SOD is the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenalin in U/min/mg protein.

2.2.4.1.3 Catalase Activity

Assay of catalase activity was carried out according to the method of Sinha (1972) based on the catalytic decomposition of hydrogen peroxide by catalase to form water. The catalase activity was calculated from the expression:

$$\text{Catalase (Units)} = \frac{\Delta \text{Abs/min} \times D \times 3}{SV \times 0.0436}$$

where: D = dilution of original sample of catalase reaction;
SV = sample volume in catalase reaction (ml).

$$\text{Catalase Activity (µmol/min/mg protein)} = \frac{\text{Units/ml}}{\text{mg/protein}}$$

2.2.4.2 In vivo Non-Enzymatic Antioxidant Assay

2.2.4.2.1 Glutathione (GSH) Level

The reduced glutathione level in the heart supernatant was determined according to the method described by Moron *et al.* (1979) based on the principle that GSH exerts its antioxidant action through its sulphydryl groups. The absorbance of the reaction mixture was read at 412 nm after 10 minutes of incubation against the reagent blank. The level of heart GSH was extrapolated from the standard calibration curve. The values were expressed as µg GSH/g sample.

2.2.4.2.2 Vitamin C Level

The vitamin C concentration in the extract was quantified using Folin-Ciocalteu's phenol reagent according to the methods described by Omaye *et al.* (1979) and Japota and Dani, (1982). Briefly, 0.5 mL of de-proteinated plasma and heart supernatant was mixed with 1.5 mL of 10% (v/v) acetic acid and 0.5 mL of Folin-Ciocalteu reagent (1:10 dilution). The mixture was incubated at room temperature for 10 minutes. The absorbance was read at 760 nm against the reagent blank. The concentration of vitamin C was obtained from the calibration curve.

2.2.4.2.3 Vitamin E Level

The total vitamin E concentration in the extract was determined according to methods of Baker and Frank (1968) and Santhosh *et al.* (2013). The analysis was based on the reduction of ferric to ferrous ion by vitamin E and the formation of red coloured complex with 2, 2'-bipyridyl. Vitamin E (0.5 mL) extracted in heptane was mixed with 0.5 mL of 2′, 2′, α-bipyridyl reagent (0.12% w/v) and 0.5 mL of ferric chloride (0.12% w/v). A known volume (0.5 mL) of the standard, Trolox (25 µg/mL) in ethanol was treated in the same way and used as a standard. The absorbance was read at 492 nm against the reaction blank. The total vitamin E was calculated from the expression:

$$\text{Concentration of Vitamin E} = \frac{\text{Absorbance of Extract} \times \text{Concentration of Standard}}{\text{Absorbance of Standard}}$$

2.2.4.2.3 Total Sugar Level

The determination of the concentration of sugar was carried out according to the method of Dubois *et al.* (1956) as slightly modified by Fasaanu *et al.* (2013) based on the formation of an orange-yellow colour when treated with phenol and concentrated sulphuric acid. Plasma or heart supernatant (0.25 mL) was made up to 1mL with distilled water after which 1 mL of 2% phenol was added. The resulting solution was mixed and incubated for 10 minutes at room temperature. Thereafter, 2 mL of concentrated sulphuric acid was added gently and carefully. The reaction mixture was mixed thoroughly and allowed to cool to room temperature. The concentration of the total sugar in the plasma was calculated using the expression:

$$\text{Concentration of sugar (µg/mL)} = \frac{\text{Absorbance of plasma} \times 250\ \text{µg/mL}}{\text{Absorbance of standard}}$$

2.2.5 Histopathological Studies

The left ventricle of the heart was fixed in 10% (v/v) formal-saline for histological study.

The fixed tissues were washed, dehydrated with alcohol and embedded in paraffin. Serial sections cut using a rotary microtome were stained with haematoxylin and eosin (H & E) (Prophet *et al.*, 1992). The slides were examined and reviewed at the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria under light microscope (Future Winjoe Photomicroscope Camera Coupled with Olympus Binocular Microscope) and photomicrograph captured at x400.

2.2.6 Statistical Analysis

The data were expressed as mean ± SD. Differences between mean values of control and treated groups were determined by One-way Analysis of Variance (ANOVA) followed by Dunnett's test using GraphPad Prism 5. Differences were considered significant if $p < 0.05$.

3.0 Results

Table 1 presents the summary of the levels of cardiac markers and weights of hearts in the control and experimental rats. In ISO treated rats, the activities of cardiac functional markers (CK-MB and LDH) were significantly increased ($p < 0.05$). Administration of the extract and propranolol significantly ($p < 0.05$) mitigated the changes. The weights of hearts of ISO-treated rats increased significantly $p < 0.05$ (41.67%) when compared with the control group (Group I). However, pre-treatment with extract and reference drug decreased the weights of rats significantly ($p < 0.05$) when compared to the ISO-treated group (Group II).

ISO altered the levels of total cholesterol (TC), triacylglycerol (TRIG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) when compared with the control group while the extract and propranolol reversed the alterations (Table 2).

Table 3 presents the summary of the treatment of rats with both extract and reference drug on the activities of some antioxidant enzymes (SOD, GPx and catalase) in the heart of the animal. There was elevation in the activities of SOD and GPx in ISO-treated rats whereas there was reduction in the activity of catalase. However, treatment of ISO-treated rats with the extract and drug brought about changes in the activities of these enzymes.

Table 4 shows the changes in the levels of plasma and heart reduced glutathione, vitamin C and vitamin E. It was observed that there was a change in the levels of the non-enzymatic antioxidants in rats treated with ISO when compared to control rats. Treatment with extract and drug restored the levels of non-enzymatic antioxidants to near normal.

The levels of plasma and heart total proteins were significantly ($p > 0.05$) reduced in ISO-treated rats when compared with the control group (Table 5). Treatment with the extract and the reference drug restored the levels of plasma and heart total protein to near normal. The levels of plasma and heart total sugars were increased in ISO-treated rats when compared with the control rats. Treatment with the extract and the drug ameliorated the changes in the levels of the plasma and heart total sugars.

Plates 1 (a-f) present the photomicrographs of sections of the heart of control and experimental rats. The microscopic observations of myocardial histoarchitecture were graded quantitatively on the basis of myonecrosis, hypertrophy and oedema. The results of the histopathological examination as revealed in the photomicrograph of stained myocardial tissue section from control rats (Plate 1a) and (Plate 1e) showed normal architecture. ISO treatment resulted in changes of heart architecture as indicated; myocardium showing massive necrosis of myofibres with oedema (black arrows) and hypertrophy (red arrows) (Plate 1b). Rats administered the extract and drug show near normal appearance of myofibres (Plates 1c, d and e).

4.0 Discussion

Plant derived chemicals are capable of terminating free radical reactions preventing oxidative damage and protecting against chronic diseases such as neurodegenerative and cardiovascular disorders (Prior *et al.*, 2005; Saikat *et al.*, 2010).

Table 1: Effects of methanol extract of *P. guineense* seeds on the cardiac maker enzymes and heart weights of rats

Group	Creatine Kinase-MB (U/L)	Lactate Dehydrogenase (U/L)	Weight of Heart (g)
I	105.67 ± 14.40	222.88 ± 58.66	0.63 ± 0.05
II	147.49 ± 10.09[a] (28.35%)	383.00 ± 32.49[a] (41.81%)	1.08 ± 0.12[a] (41.67%)
III	82.55 ± 11.91[b] (44.03%)	256.86 ± 47.59[b] (32.93%)	0.94 ± 0.14 (12.96%)
IV	82.55 ± 18.83[b] (44.03%)	283.26 ± 38.62[b] (26.05%)	0.71 ± 0.10[b] (34.26%)
V	115.57 ± 18.68 (8.57%)	294.86 ± 45.52 (24.41%)	0.80 ± 0.06 (25.93%)
VI	113.92 ± 7.01 (22.76%)	279.80 ± 10.47[b] (26.94%)	0.74 ± 0.21 (31.48%)

Results were expressed as the mean ±SD, n=5; [a]$p < 0.05$ statistically significant when compared with normal control; [b]$p < 0.05$ statistically significant when compared with ISO-treated group
Grp I = Normal control; Grp II = ISO treated; Grp III = MEPG (125 mg/kg bwt + ISO; Grp IV = MEPG (250 mg/kg bwt + ISO; Grp V = MEPG (250 mg/kg bwt); Grp VI = Propranolol (1.8 mg/kg bwt + ISO).

Table 2: Effect of Effects of methanol extract of *P. guineense* seeds on lipid profile of rats

Group	TC (mmol/L)	TRIG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	VLDL-C (mmol/L)
I	0.67 ± 0.18	0.52 ± 0.14	0.42 ± 0.04	0.26 ± 0.19	0.39 ± 0.13
II	0.94 ± 0.18 (28.72%)	0.26 ± 0.06 (50.00%)	0.44 ± 0.04 (4.55%)	0.34 ± 0.14 (23.53%)	0.41 ± 0.08 (4.88%)
III	0.80 ± 0.02 (17.50%)	0.46 ± 0.33 (43.48%)	0.49 ± 0.05 (10.20%)	0.31 ± 0.11 (9.68%)	0.44 ± 0.11 (6.82%)
IV	0.82 ± 0.16 (14.63%)	0.37 ± 0.02 (29.73%)	0.46 ± 0.04 (4.35%)	0.36 ± 0.13 (5.56%)	0.35 ± 0.21 (14.63%)
V	0.62 ± 0.16 (8.06%)	0.54 ± 0.18 (3.70%)	0.43 ± 0.03 (2.33%)	0.13 ± 0.01 (50.00%)	0.28 ± 0.07 (28.00%)
VI	0.69 ± 0.16 (26.60%)	0.42 ± 0.08 (38.10)	0.40 ± 0.07 (9.09%)	0.16 ± 0.10 (38.46%)	0.31 ± 0.07 (24.39%)

Results were expressed as the mean ±SD, n=5; [a]$p < 0.05$ statistically significant when compared with normal control; [b]$p < 0.05$ statistically significant when compared with ISO-treated group
Grp I = Normal control; Grp II = ISO treated; Grp III = MEPG (125 mg/kg bwt + ISO; Grp IV = MEPG (250 mg/kg bwt + ISO; Grp V = MEPG (250 mg/kg bwt); Grp VI = Propranolol (1.8 mg/kg bwt + ISO). TC- Total Cholesterol; TRIG- Triacylglycerol; HDL-C- High-density Lipoprotein Cholesterol; LDL-C- Low-density Lipoprotein Cholesterol; VLDL-C- Very Low-density Lipoprotein Cholesterol.

Table 3: Effects of methanol extract of *P. guineense* seeds on enzymatic antioxidants

Group	SOD (U/min/mg protein)	GPx (µmol/min/mol/mg protein)	Catalase (µmol/min/mg protein)
I	0.12 ± 0.02	0.26 ± 0.03	1.06 ± 0.19
II	0.17 ± 0.03 (29.41%)	0.32 ± 0.08 (18.75%)	0.58 ± 0.34 (14.74%)
III	0.22 ± 0.04 (22.73%)	0.29 ± 0.05 (9.38%)	0.68 ± 0.25 (11.59%)
IV	0.29 ± 0.08 (41.38%)	0.30 ± 0.03 (6.25%)	1.90 ± 0.03[b] (53.85%)
V	0.30 ± 0.17[a] (60.00%)	0.29 ± 0.04 (10.34%)	1.05 ± 0.30 (52.85%)
VI	0.20 ± 0.02 (15.00%)	0.33 ± 0.04 (3.03%)	1.82 ± 0.54[b] (13.88%)

Results were expressed as the mean ±SD, n=5; [a]$p < 0.05$ statistically significant when compared with normal control; [b]$p < 0.05$ statistically significant when compared with ISO-treated group
Grp I=Normal control; Grp II=ISO treated; Grp III=MEPG (125 mg/kg bwt + ISO); Grp IV=MEPG (250 mg/kg bwt + ISO); Grp V=MEPG (250 mg/kg bwt); Grp VI=Propranolol (1.8 mg/kg bwt + ISO).

Table 4: Effects of methanol extract of *P. guineense* seeds on non-enzymatic antioxidants of rats

Groups	GSH (µg/g)	Plasma Vit. C (mg/dL)	Heart Vit. C (mg/g)	Plasma Vit. E (µg/dL)	Heart Vit. E (µg/g)
I	5.96 ± 0.49	10.75 ± 1.16	4.80 ± 0.52	12.63 ± 0.10	1.45 ± 0.04
II	6.99 ± 0.97 (14.74%)	11.9 ± 0.66 (9.66%)	5.61 ± 0.50 (14.44%)	12.38 ± 0.24 (1.98%)	1.47 ± 0.05 (1.36%)
III	6.18 ± 0.37 (11.59%)	10.68 ± 0.26 (10.25%)	5.73 ± 0.64 (2.09%)	12.72 ± 0.79 (2.67%)	1.42 ± 0.04 (3.40%)
IV	3.22 ± 0.37 (53.93%)	10.32 ± 0.94[b] (13.28%)	6.89 ± 0.11[b] (18.58%)	13.06 ± 0.26 (5.21%)	1.44 ± 0.05 (2.04%)
V	2.81 ± 0.30[a] (52.85%)	10.88 ± 0.71 (1.19%)	7.20 ± 0.34[a] (33.33%)	12.83 ± 0.34 (9.44%)	1.44 ± 0.03 (3.40%)
VI	6.02 ± 0.30 (13.88%)	10.30 ± 0.64[b] (13.45%)	6.58 ± 0.67 (14.74%)	13.67 ± 0.49 (1.56%)	1.42 ± 0.03 (0.69%)

Results were expressed as the mean ±SD, n=5; [a]$p < 0.05$ statistically significant when compared with normal control; [b]$p < 0.05$ statistically significant when compared with ISO-treated group; Grp I = Normal control; Grp II = ISO treated; Grp III = MEPG (125 mg/kg bwt + ISO; Grp IV = MEPG (250 mg/kg bwt + ISO; Grp V = MEPG (250 mg/kg bwt); Grp VI = Propranolol (1.8 mg/kg bwt + ISO).

Table 5: Effects of methanol extract of *P. guineense* seeds on the levels of plasma and heart total proteins and sugars of rats

Groups	Plasma Protein (g/dL)	Heart Protein (mg/g)	Plasma Sugar (mg/L)	Heart Sugar (mg/g)
I	7.46 ± 1.85	8.37 ± 0.59	1.83 ± 0.07	2.20 ± 0.40
II	5.23 ± 0.22[a] (29.89%)	7.22 ± 0.82[a] (13.74%)	1.99 ± 0.23 (8.04%)	1.59 ± 0.34 (27.73%)
III	6.60 ± 1.56 (20.76%)	8.67 ± 0.56[b] (16.72%)	2.31 ± 0.29[b] (13.85%)	2.26 ± 0.57 (29.65%)
IV	7.30 ± 1.16[b] (28.36%)	8.35 ± 0.43[b] (13.53%)	2.24 ± 0.24[b] (11.16%)	2.54 ± 0.30[b] (37.40%)
V	8.12 ± 0.68 (35.59%)	8.82 ± 0.53 (18.14%)	2.54 ± 0.39[a] (27.95%)	2.41 ± 0.43 (8.71%)
VI	6.55 ± 0.71 (20.15%)	8.27 ± 0.32 (12.70%)	1.66 ± 0.07 (16.58%)	1.70 ± 0.07 (6.47%)

Results were expressed as the mean ±SD, n=5; [a]$p \leq 0.05$ statistically significant when compared with normal control; [b]$p < 0.05$ statistically significant when compared with ISO-treated group
Grp I = Normal control; Grp II = ISO treated; Grp III = MEPG (125 mg/kg bwt + ISO; Grp IV = MEPG (250 mg/kg bwt + ISO; Grp V = MEPG (250 mg/kg bwt); Grp VI = Propranolol (1.8 mg/kg bwt + ISO).

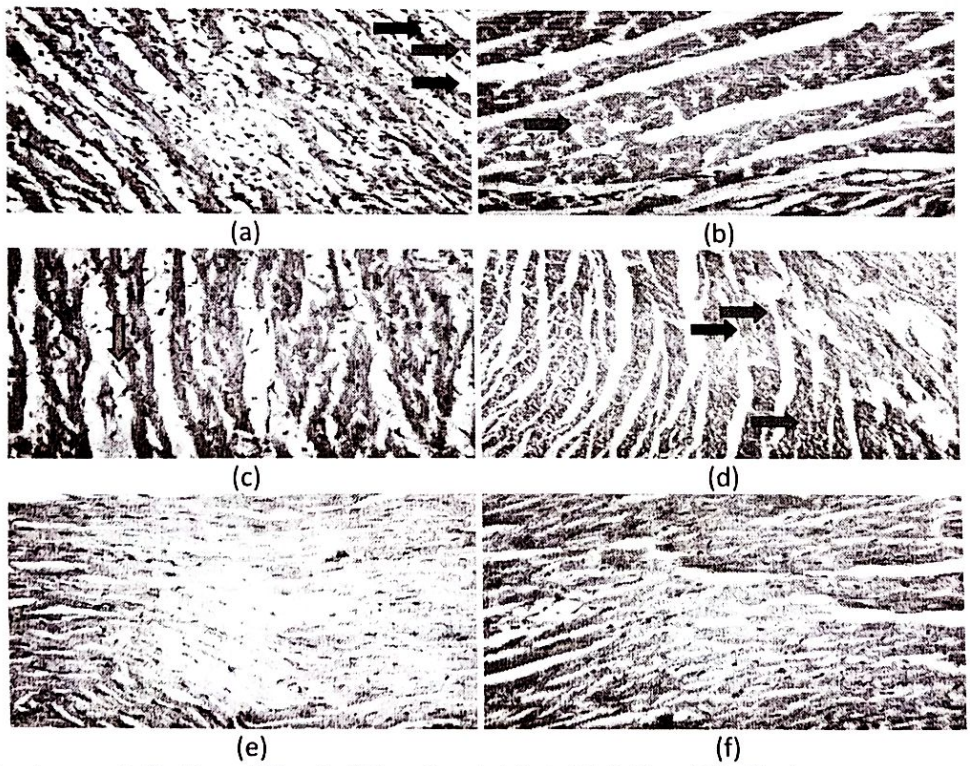

Plate 1: Photomicroscopic Sections of Heart of Experimental Rats (H & E; x 400). Black arrow represents necrosis; Red arrow represents hypertrophy; Green represents dystrosphy. **A:** Section of the heart of the control group (Group I); **B:** Section of the heart of rats + Isoproterenol (ISO) (80 mg/kg bwt) (Group II); **C:** Section of the heart of rats + extract (125 mg/kg bwt) + ISO (Group III); **D:** Section of the heart of rats + extract (250 mg/kg bwt) + ISO (Group IV); **E:** Section of the heart of rats + extract (250 mg/kg bwt) (Group V); **F:** Section of the heart of rats + Propranolol (1.8 mg/kg bwt) + ISO (Group VI).

Phenolics are ubiquitous groups of plant metabolites that exhibit a wide range of physiological properties including anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective and vasodilatory effects (Goswami *et al.*, 2013), improvement of endothelial function and cardiovascular protection (Aiyegroro and Okoh, 2010). Previous and preliminary studies on the plant revealed that the plant is rich in phenolics and its oil exhibit potent and appreciable antioxidant activities (Ekpo *et al.*, 2013; Etim *et al.*, 2013; Tankam and Ito, 2013; Opara, 2014; Oyemitan *et al.*, 2014).

The results further revealed that as a result of ISO administration, the weights of the hearts of the animals increased significantly, although the body weight remained relatively unchanged when compared with the control group. The increase in the weights of the heart might be attributed to increased water accumulation in oedematous intramuscular spaces in cardiac tissue and extensive necrosis of cardiac muscle fibres followed by the invasion of damaged tissues by inflammatory cells (Patel *et al.*, 2010). This was also confirmed by histopathological examinations. Patel *et al.* (2010) and Khalil *et al.* (2015) reported that myocardial function could be reduced by approximately 10% with a 1% increase in myocardial water content. It was observed that pre-treatment with extract however, reduced the weights of the hearts of the animals to near normal, which is indicative of its protective effect on the myocardium. The result also showed that the protection on the myocardium by the MEPG was concentration-dependent.

Myocardial cells contain variety of cardiac enzymes (creatine kinase-MB and lactate dehydrogenases) which are employed as diagnostic markers of myocardial infarction (Nagaraja *et al.*, 2011). Once myocardial cells are damaged or destroyed, the cardiac membrane becomes permeable or ruptures, resulting in leakage of cytosolic enzymes into the bloodstream with concomitant increases in their plasma concentrations (Upaganlawar *et al.*, 2009; Wang *et al.*, 2009; Prabha *et al.*, 2014). Komolafe *et al.* (2013) reported that detection of cardiac injury could be done by determining the serum/plasma levels of known cardiac marker enzymes such as creatine-MB, LDH and serum/plasma lipid profile. In the present study, ISO administration caused marked elevations of the plasma cardiac marker enzyme activities (CK-MB and LDH). However, pre-treatment of the rats with seed extract at the two dose levels (125 and 250 mg/kg bwt) significantly lowered the ISO-induced elevation of plasma levels of the cardiac biomarkers. The extract exhibited more potency than the reference drug as it produced better lowering ability of the marker enzymes. The levels of these cellular enzymes present in the blood are directly related to the intactness of the plasma membrane of the cardiac cells (Chen *et al.*, 2008). Thus, the inhibition of ISO-induced elevation of marker enzymes in plasma (CK-MB and LDH) by MEPG could be due to its action on maintaining cardiac membrane integrity. The results of this investigation are consistent with earlier observations that administration of ISO elicited the release of cardiac markers and that cardioprotective compounds cause reduction of the altered biomarkers (Nagaraja *et al.*, 2011; Radhika *et al.*, 2013; Prabha *et al.*, 2014; Khalil *et al.*, 2015).

Wilson *et al.* (1998) reported that some of the biochemical indices used in the prediction of cardiovascular disease risk could be alterations in lipid metabolism and serum/plasma levels of cholesterol, triacylglycerols and LDL-C. Also, lower functional HDL-C and elevated LDL-C levels which promote atherosclerotic progression are associated with cardiovascular diseases (Crowwell and Otvos, 2004). In this study, increased concentrations of TC, TRIG, LDL and VLDL, and reduced concentration of HDL were observed. These alterations in the lipid profile by ISO-induced cardiotoxicity could be attributed to enhanced biogenesis of lipids by the cardiac cAMP cascade (Paritha and Shyamala, 1997). MEPG modulates lipid profile of rats following administration of ISO, portraying cardioprotective ability of the extract, taking into account the role of dyslipidaemia in the origin and progression of many cardiovascular diseases (Komolafe *et al.*, 2013).

Proteins, synthesized in the liver, are mainly involved in the architecture of the cell, and the quantity depends on the rate of synthesis and degradation (Radhika *et al.*, 2013). The major

plasma/serum proteins include globulin, albumin and conjugated proteins like glycoprotein (Chawla, 1999). Soluble sugars are synthesized in response to stress (Peshev and Van den Ende 2013). Keunen *et al.* (2013) reported that excess soluble sugar production and sugar availability determine the rate of reducing power production contributing to H_2O_2 scavenging by feeding the NADPH-producing OPP pathway. In the present study, there was significant decrease in the total protein levels in the plasma and heart of the ISO-treated rats when compared with the normal. Rekha and Saleem (2008) observed that a decrease in serum protein is usually as a fall in albumin or sometimes γ-globulin. Radhika *et al.* (2013) reported that ISO-induced myocardial infarction (MI) is a free radical mediated tissue damage which may lead to the production of more oxygen and hydrogen peroxide ions binding with albumin and thus destroying it. Pre-treatment with the extract significantly increased the levels of total proteins following administration of ISO and compared favourably with the reference drug. The treatment with the extract alone showed improved levels of total protein in both plasma and heart, indicating a balance in protein metabolism. This result indicates that the extract has the ability to protect the heart from oxidative damage.

Also, there was a decrease in the heart total sugar but increase in plasma sugar in the rats treated with ISO which might be contributing to H_2O_2 scavenging (Keunen *et al.*, 2013). The pre-treatment with the extract increased the levels of the plasma total sugar while the heart total sugar was restored to near normal.

Oxidative stress has been reported to play vital role in the development and progression of MI and heart failure (Bryne *et al.*, 2003). There has been a direct link between activities of antioxidants and the prevention of reactive oxygen species (ROS) in ISO-administered rats (Gayathri *et al.*, 2010; Prabha *et al.*, 2014). The intracellular antioxidant system comprises different free radical scavenging antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) which constitute the first line of cellular antioxidant defense enzymes along with some non-enzyme antioxidants like reduced glutathione (GSH), vitamins C and E (Mudagal *et al.*, 2011).

Reduced glutathione (GSH) is a ubiquitous antioxidant and an essential bio-factor synthesized in all living cells, protecting the cells against free radical mediated injury caused by drugs (Nagaraja *et al.*, 2011). It forms an important substrate for several other antioxidant enzymes. It is closely interlinked with vitamin C and vitamin E playing a key role in protecting the cell membrane from oxidation (Pari *et al.*, 2015). The GSH plays a major role in restoring other free radical scavengers and antioxidants such as vitamin C and vitamin E (Gayathri *et al.*, 2010; Nagaraja *et al.*, 2011; Pari *et al.*, 2015). Gayathri *et al.* (2010) reported that vitamin C can prevent the oxidation of lipids by trapping water soluble peroxyl radicals before their diffusion into lipid membranes. Vitamin E is a fat-soluble vitamin with its main function being to protect against lipid peroxidation and is responsible for high rate of reaction with lipid radicals and able to scavenge superoxide radicals (Gayathri *et al.*, 2010; Pari *et al.*, 2015). In the present study, increased GSH, vitamin C and vitamin E levels were observed which might be a response to the increased generation of ROS (Pari *et al.*, 2015). Treatment with the extract restored the levels of the non-antioxidants to normal probably by minimising the usage of these antioxidants (Gayathri *et al.* 2010; Pari *et al.*, 2015).

Superoxide dismutase (SOD), an endogenous radical scavenging antioxidant enzyme, catalyzes the removal of superoxide radicals (Khalil *et al.*, 2015) generated during ISO metabolism, to H_2O_2 which is a substrate for GPx and catalase enzymes. GPx is a seleno-enzyme, which catalyzes the GSH dependent reduction of H_2O_2 and other peroxides and protects against oxidative damage (Ansil *et al.*, 2011). Catalase is an endogenous radical detoxifying enzyme which catalyzes the decomposition of hydrogen peroxide generated by SOD activities to water (Lobo *et al.*, 2010). In the present study, perturbed activities of catalase, GPx and SOD were found in heart of ISO-treated rats. The extract offers protection against oxidative damage due to enhanced antioxidant activity (Gayathri *et al.*, 2010; Prabha *et al.*, 2014; Khalil *et al.*, 2015; Pari *et al.*, 2015).

Histopathological observations on the heart showed that ISO induced myocardial necrosis with oedema, hypertrophy and separation of cardiac muscle fibres with inflammatory cell infiltration. Pre-treated rats with extract showed improved cardiac muscle fibre architecture with reduced inflammatory infiltration. Treatment with the extract alone showed improved cardiac histoarchitecture, indicating the extract did not elicit any adverse effect on the myocardium. Similar findings were reported in ISO-treated rats treated with Tualang honey (Khalil *et al.*, 2015), *Gardenia gummifera* (Prabha *et al.*, 2014) and lemon grass (Gayathri *et al.*, 2010).

Conclusion

The study demonstrates the protective effect of *P. guineense* seed extract in ISO-induced myocardial infarction in rats. The cardioprotective potential might be related to the antioxidant and antihyperlipidaemic activities of the plant. Thus, *Piper guineense* could be regarded as a promising medicinal plant with cardioprotective activity.

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