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Cardioprotective effects and antioxidant status of *Andrographis paniculata* in isoproterenol-induced myocardial infarction in rats

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Background: Myocardial infarction has been regarded as one of the fastest killer diseases of modern-day man.

Aim: The protective effect of *Andrographis paniculata* on isoproterenol (ISO)-induced myocardial infarction in rats was investigated.

Setting: The study was carried out in a laboratory setting.

Methods: Animals were randomly divided into six groups of seven animals per group, and the treatment was as follows: normal control received normal saline for 9 days, isoproterenol group; three extract-treated groups in pre-treatment phase and an extract-treated group in post-treatment phase. The doses were given at 100, 200 and 400 mg/kg body weight for pre-treatment phase respectively while 200 mg/kg dose was given to the post-treatment phase group. Blood and heart tissues were collected for biochemical assays, haematological and histological analyses.

Results: Myocardial infarction was recorded in ISO group but was corrected by the extracts in both pre-treatment and post-treatment phases. The ISO group experienced a significant decrease in antioxidant parameters, whereas the extract at all doses caused a significant increase in the activities of in these parameters. The extract caused a significant decrease in malondialdehyde content and hydrogen peroxide generation, whereas reverse was the case for the ISO group. Although no significant histopathological changes were recorded for the extract, the ISO group showed marked histopathological changes. ISO caused higher expressions of cardiac C-reactive protein (CRP) and CTnI and decreased the expressions of IL-10β; but this was the opposite for the extract.

Conclusion: The ethanol leaf extract of *A. paniculata* significantly exhibits cardioprotective effects.

Introduction

Catecholamines are released under the conditions of stress; hence, they are also administered in circumstances of cardiac stress to sustain blood pressure and cardiac function in patients. These agents are important regulators of myocardial contractility and metabolism, but excess amounts of catecholamines are responsible for damage at cellular level as observed in clinical conditions such as acute coronary insufficiency, transient myocardial hypoxia, angina and subendocardial infarct. Because catecholamines generate reactive oxygen species (ROS), they thus contribute to oxidative stress (Yogeeta et al. 2006; Hussein 2015). ROS can initiate lipid peroxidation reactions and propagate cell membrane damage (Chen et al. 2000). Isoproterenol [1-(30, 40-dihydroxy phenyl)-2-isopropyl amino ethanol hydrochloride] (ISO) is a synthetic catecholamine, which acts as a β-adrenergic agonist. It has been found to produce stress in the myocardium leading to severe toxicity in the myocardium, thus, resulting in depletion of energy reserve of cardiac muscle cells. It is also known to cause complex biochemical and structural changes leading to cell damage and necrosis (Yogeeta et al. 2006). Some of the mechanisms proposed to explain ISO-induced damage to cardiac myocytes include coronary hypotension, energy depletion, hypoxia, calcium overload and excessive production of free radicals as a result of catecholamine autoxidation (Adameova, Abdellatif & Dhalla 2009; Rona et al. 1959). To study the beneficial effects of many drugs on cardiac function, ISO-induced myocardial damage was considered as one of the most widely used experimental models (Rona 1985). Several natural products are reported to prevent ISO-induced myocardial infarction (MI) because of their antioxidant activity (Chen et al. 2000; Murugesan et al. 2012; Upaganlawar & Balaraman 2011; Upaganlawar, Gandhi & Balaraman 2011). The antioxidant

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activities of *A. paniculata* and its constituents have been reported (Zhang & Tan 2000; Verma & Vinayak 2008).

Andrographis paniculata (Burm) Wall ex Nees (Acanthaceae) is an important medicinal plant that is widely used globally (Hossain et al. 2014). The pharmacological potentials of *A. paniculata* such as anti-inflammatory (Sheeja & Kuttan 2012), immunostimulatory (Sheeja & Kuttan 2012), hepatoprotective (Trivedi & Rawal 2001), antihyperglycemic (Reyes et al. 2006), antioxidant (Akowuah, Zhari & Mariam 2008; Kamdem, Sang & Ho 2002; Singh, Banerjee & Rao 2001) and cardiovascular activities (Zhang & Tan 1997) have been demonstrated. The major phytoconstituent of this plant is andrographolide, a labdane diterpenoid. Other bioactive secondary metabolites of the plant such as diterpenoids, diterpene glycosides, lactones, flavonoids and flavonoid glycosides are also present and are involved in antidiabetic, hepatoprotective, antioxidant, anti-inflammatory and diverse other therapeutically interesting bioactivities (Akbar 2011; Jayakumar et al. 2013; Parixit et al. 2012).

In this study, the ethanol leaf extract of *A. paniculata* was used to evaluate the cardioprotective effect of this plant using its antioxidant properties as well as haemodynamics, histopathologic and immunohistochemical changes.

Materials and methods

Plant collection and extract preparation

The leaves of the plant *A. paniculata* were collected from the University of Ibadan Botanical Garden. The plants were identified and authenticated with voucher numbers: UI-UIH 2846 by herbarium curator, Department of Botany, Faculty of Science, University of Ibadan. The leaves were cleaned with distilled water and air dried in a well-ventilated shady room. The dried leaves were grinded to powder using a blender. The grinded powder was extracted in cold ethanol in a screw-capped flask for 72 h and shaken at room temperature. The solvent was filtered, squeezed off and evaporated off under reduced pressure in a rotatory evaporator at 40 °C to obtain semi-solid crude extract which was stored at 4 °C. The stored ethanol extract of *A. paniculata* (EEAP) was then used for the study.

Chemicals and reagents

Isoproterenol, Biuret reagent, hydrogen peroxide, hydrochloric acid, sulphuric acid, xylenol orange, potassium dichromate, O-diasinidine, sodium potassium tartrate, copper sulphate, ethanol, sodium azide, 2-dichloro-4-nitrobenzene (CDNB), Griess reagent, phosphoric acid, sodium hydroxide, N-(1-naphthyl)ethylenediamine, sulphanilamide, distilled water, phosphate buffer saline, creatinine reagent, copper sulphate, trichloroacetate, reduced glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), ammonium ferrous sulphate, glacial acetic acid, potassium iodide, sorbitol, Ellman's reagent (DTNB), ethanol and urea reagent. All chemicals and drugs used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

Experimental animals

All experiments and protocols described in this study were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC). Forty-two male Wistar rats weighing 120 g – 150 g were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine for the study. These animals were allowed free access to standard rat pellets and fresh water *ad libitum* in the Animal House Unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, where they were housed with a 12-h light duration. Preconditioning of the rats was performed for 2 weeks before the commencement of the experiment.

Cardioprotective study

The animals were randomly divided into six groups of seven animals each, and the treatment was as follows: the control (group A) received normal saline for 9 days, group B animals received normal saline for 7 days and thereafter isoproterenol (ISO) at 85 mg/kg on days 8 and 9. Groups C, D and E animals, on the other hand, were pre-treated with the ethanol leaf extract of *A. paniculata* at 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively, for 7 days and thereafter received ISO on days 8 and 9. Group F animals were treated with ISO on days 1 and 2 and thereafter received 200 mg/kg of the extract for the remaining 7 days.

Blood pressure measurement

Blood pressure measurement of all the animals was carried out on day 10. Baseline cardiovascular parameters were collected prior to the commencement of the experiment. The equipment used was a non-invasive tail cuff BP monitor, the 6-channel CODA blood pressure monitor for rats and mice. Blood pressure parameters including the systolic, diastolic and mean arterial blood pressure parameters were determined indirectly in non-anaesthetised rats by tail plethysmography with the use of an electrosphygomanometer (CODA, Kent Scientific, USA). The average of at least nine most consistent readings, taken in the quiescent state, following acclimatisation, was recorded per animal.

Blood sample collection

At the end of the experimental period, blood samples were collected for serum chemistry before the rats were sacrificed by cervical dislocation. The serum in plain bottles was rapidly centrifuged at 4000 revolutions per minute (rpm) for 15 min and processed for determination of serum myeloperoxidase, total protein, aspartate transaminase (AST), alanine aminotransferase (ALT), and nitric oxide (NO). The heart of each rat was carefully removed and homogenised on ice and then used to assay for some oxidative stress markers and antioxidant parameters.

Preparation of tissue homogenate

The heart tissues of the rats were harvested on ice, rinsed with normal saline and homogenised in aqueous potassium buffer (0.1 M, pH 7.4), and the homogenate was centrifuged at 12000 rpm (4 °C) for 15 min to obtain the supernatant fraction.

Biochemical assays

Determination of the protein concentrations of the various samples was by Biuret method as described by Gornal, Bardawill and David (1949). To prevent precipitation of Cu^{2+} ions, cuprous oxide potassium iodide was added to the reagent. The method of Beutler and Kelly (1963) was used to determine the concentration of reduced GSH, whereas glutathione peroxidase (GPx) activity was measured by the method of Rotruck et al. (1973). In this case, hydrogen peroxide was used as substrate to oxidise reduced GSH to oxidised glutathione (GSSG). The estimation of glutathione-S-transferase (GST) was done by the method of Habig, Pabst and Jacoby (1974) using 1-chloro-2, 4-dinitrobenzene as substrate. Superoxide dismutase (SOD) assay, on the other hand, was carried out by the method of Misra and Fridovich (1972) with slight modification. The MDA content was measured in the heart as an index of lipid peroxidation as described by Varshney and Kale (1990). The method of Wolff (1994) was used to measure hydrogen peroxide generation, whereas sulfhydryl (Thiol) content determination was performed by the method of Ellman (1959). The quantification of NO was carried out as previously described by Olaleye, Adaramoye and Erigbali (2007).

Histopathology

Small slices of the heart were collected in 10% buffered formalin for proper fixation, and after the tissues have been processed and embedded in paraffin wax, sections of about 5 µm – 6 µm thick were made and stained with haematoxylin and eosin for histopathological examination (Drury, Wallington & Cancerson 1976).

Immunohistochemistry of cardiac troponin-1, cardiac C-reactive protein and Interleukin-10 (IL-10)

The heart tissues obtained from buffered formalin perfused rats were paraffin embedded and then used for immunohistochemistry. Paraffin sections were melted at 60 °C in the oven, but the dewaxing of the samples in xylene was followed by passage through decreasing concentrations of ethanol (100% – 80%). Peroxidase quenching in 3% H_2O_2/methanol was carried out with subsequent antigen retrieval performed by microwave heating in 0.01 M citrate buffer (pH 6.0) to boil. All the sections were blocked in normal goat serum (10%, HistoMark®, KPL, Gaithersburg, MD, USA) and probed with cardiac troponin-1 (CTnI), cardiac C-reactive protein (CRP) antibody and IL-10 (Abclonal®), 1:375 for 16 h in a refrigerator. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 µg/mL) secondary antibody and, subsequently, streptavidin peroxidase (Horse Radish Peroxidase-streptavidin) according to the manufacturer's protocol (HistoMark®, KPL, Gaithersburg, MD, USA).

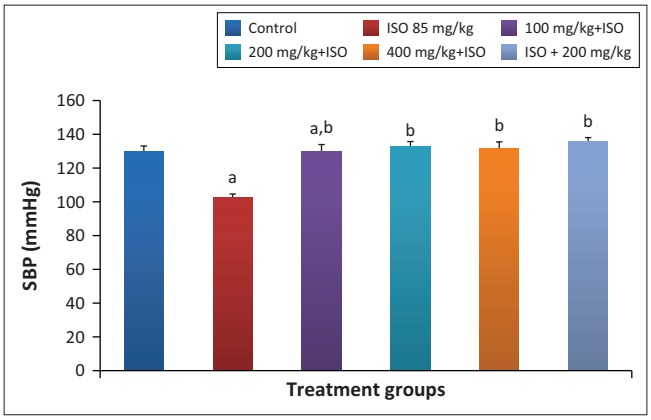
Diaminobenzidine (DAB, Amresco®, USA) was used to enhance the reaction product for 6–10 min and counterstained with high-definition haematoxylin (Enzo®, NY, USA), and was thereafter dehydrated in ethanol. Once the slides were covered with cover slips, they were sealed with resinous solution. The immunoreactive positive expression of CTnI, CRP and IL-10 intensive regions was viewed starting from low magnification on each slice and then with 400× magnifications using a photo microscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

Statistical analysis

All values were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's *post hoc* test using Graph pad prism 5.0 was also performed, with $p < 0.05$ considered statistically significant.

Results

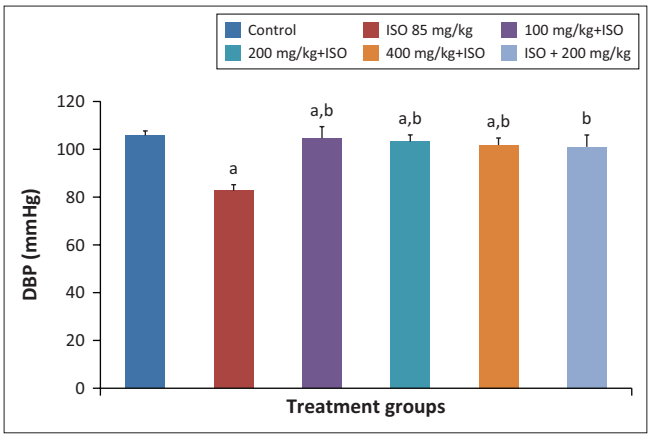
In this study, MI as seen by significant decrease in systolic, diastolic and mean arterial pressure was recorded in the ISO group but was corrected by the extracts in both pre-treatment and post-treatment phases (Figures 1–3). There was a significant increase in white blood cells (WBC) in the ISO group (Table 1). The ISO group showed a significant decrease in antioxidant parameters, whereas pre-treatment (100 mg/kg) and post-treatment with the extract caused a significant increase in the levels of SOD, GPx, GST and GSH (Figures 4–7). The extract caused a significant decrease in MDA content, MPO and H_2O_2 generation, whereas reverse was the case for group B animals (Figures 8 and 9). The extract at all doses caused a significant increase in the levels of protein



The results showed the effect of ethanol extract of *Andrographis paniculata* (EEAP) on SBP where values are presented as mean ± standard deviation.

a, indicates significant reduction (α < 0.05) when compared with control (Grp A); b, indicates significant increase (α < 0.05) when compared with ISO treated only (Grp B). Grp A (control), Grp B (ISO treated only), Grp C (100 mg/kg AP + ISO), Grp D (200 mg/kg AP + ISO), Grp E (400 mg/kg AP + ISO) and Grp F (ISO +200 mg/kg AP post-treated). AP, *Andrographis paniculata*.

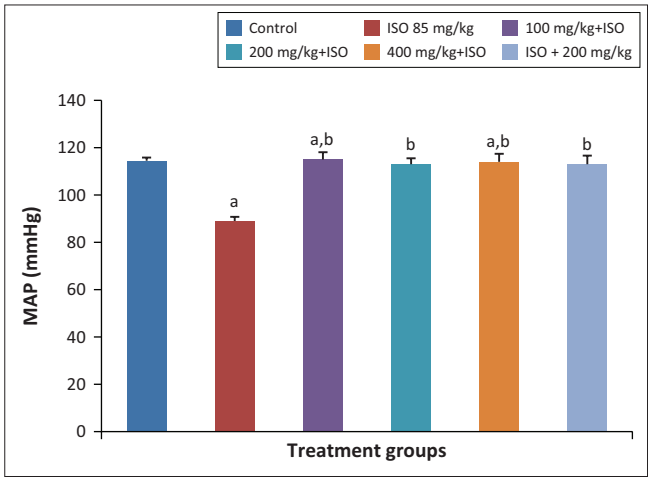
FIGURE 1: Effect of the ethanolic leaf extract of *Andrographis paniculata* on systolic blood pressure (SBP) in isoproterenol-induced myocardial infarction in rats.



The results showed the effect of ethanol extract of *Andrographis paniculata* (EEAP) on DBP where values are presented as mean ± standard deviation.

a, indicates significant reduction (α < 0.05) when compared with control (Grp A); b, indicates significant increase (α < 0.05) when compared with ISO treated only (Grp B). Grp A (control), Grp B (ISO treated only), Grp C (100 mg/kg AP + ISO), Grp D (200 mg/kg AP + ISO), Grp E (400 mg/kg AP + ISO) and Grp F (ISO +200 mg/kg AP post-treated). AP, *Andrographis paniculata*.

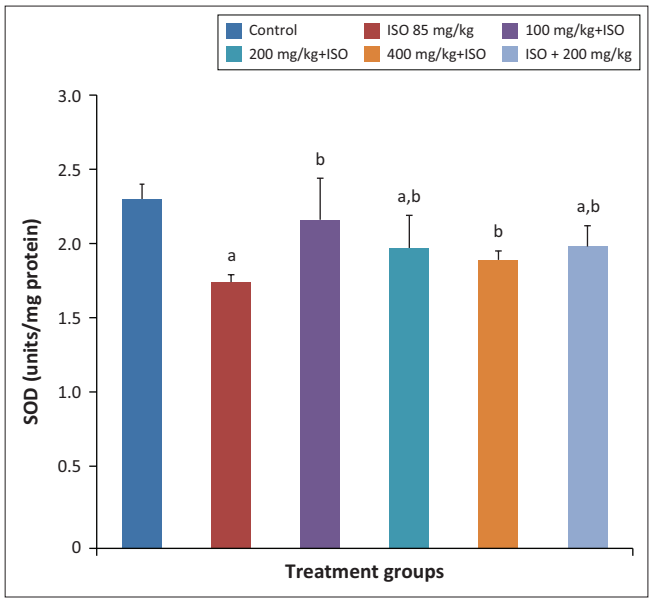
FIGURE 2: Effect of the ethanolic leaf extract of *Andrographis paniculata* on diastolic blood pressure (DBP) in isoproterenol-induced myocardial infarction using rats as a model.



The results showed the effect of ethanol extract of *Andrographis paniculata* (EEAP) on MAP where values are presented as mean ± standard deviation.

a, indicates significant reduction (α < 0.05) when compared with control (Grp A); b, indicates significant increase (α < 0.05) when compared with ISO treated only (Grp B). Grp A (control), Grp B (ISO treated only), Grp C (100 mg/kg AP + ISO), Grp D (200 mg/kg AP + ISO), Grp E (400 mg/kg AP + ISO) and Grp F (ISO +200 mg/kg AP post-treated). AP, *Andrographis paniculata*.

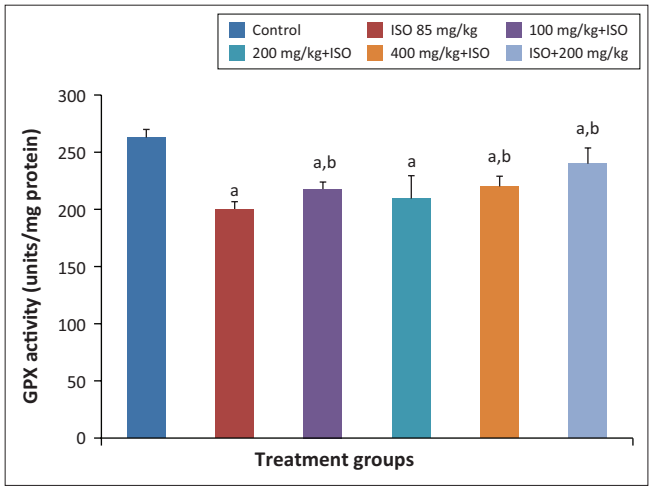
FIGURE 3: Effect of the ethanolic leaf extract of *Andrographis paniculata* on mean arterial pressure (MAP) in isoproterenol-induced myocardial infarction using rats as a model.



Values are presented as mean ± standard deviation.

a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 4: Effect of the ethanolic extract of *Andrographis paniculata* on superoxide dismutase enzyme in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



Values are presented as mean ± standard deviation.

a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 5: Effect of the ethanolic extract of *Andrographis paniculata* on glutathione peroxidase enzyme in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).

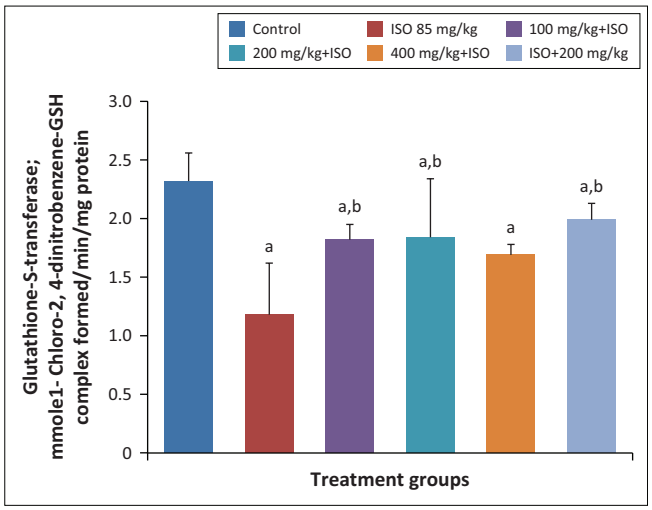
TABLE 1: Effects of the ethanolic leaf extract of *Andrographis paniculata* on haemogram in isoproterenol-induced myocardial infarction using rats as a model.

	Groupings		Pre-treated groups			Post-treated groups
Parameters	**Control**	**ISO**	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	**200 mg/kg**
RBC (×10^{12}/L)	4.75 ± 0.90	4.96 ± 0.43	5.51 ± 1.05	5.86 ± 0.47[a,b]	5.08 ± 0.59	6.71 ± 0.34[b]
WBC (10^3/μL)	5.47 ± 0.38	6.71 ± 1.13[a]	5.95 ± 1.64	7.50 ± 1.64[a]	6.70 ± 1.29[a]	7.73 ± 1.09[a]
HB (g/dL)	13.33 ± 1.40	15.15 ± 1.84	15.25 ± 1.71	15.24 ± 1.83	13.64 ± 1.61	14.95 ± 1.37
PCV (%)	45.75 ± 4.65	54.25 ± 4.25[a]	52.75 ± 1.89[a]	49.25 ± 3.86[b]	47.00 ± 2.58[b]	49.67 ± 0.58[a]
MCV (fl)	96.32 ± 9.03	109.37 ± 30.12	95.73 ± 14.08	84.04 ± 16.31	92.52 ± 9.73	74.02 ± 6.83[b]
MCH (pg)	28.06 ± 3.48	30.54 ± 8.08	27.68 ± 5.30	26.01 ± 5.24	26.85 ± 2.56	22.28 ± 3.17
MCHC (%)	29.14 ± 2.05	27.93 ± 2.38	28.91 ± 4.46	30.94 ± 2.81	29.02 ± 1.32	30.09 ± 1.68

Note: The results showed the effect of ethanol extract of *Andrographis paniculata* (EEAP) on haemogram where values are presented as mean ± standard deviation.

RBC, Red blood cells; WBC, white blood cells; ISO, isoproterenol; HB, Haemoglobin; PCV, Packed Cell Volume; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration.

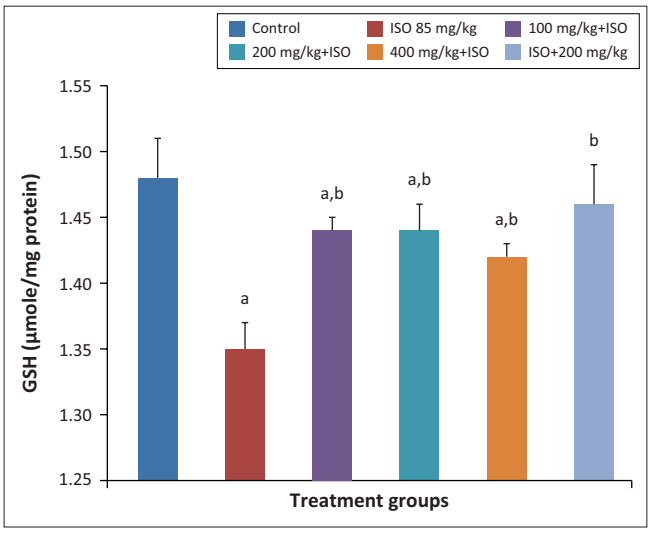
a, indicates significant different (α < 0.05) when compared with control (Grp A); b, indicates significant different (α < 0.05) when compared with ISO treated only (Grp B). Grp A (control), Grp B (ISO treated only), Grp C (100 mg/kg AP + ISO), Grp D (200 mg/kg AP + ISO), Grp E (400 mg/kg AP + ISO) and Grp F (ISO +200 mg/kg AP post-treated). AP, *Andrographis paniculata*.



Values are presented as mean ± standard deviation.

a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 6: Effect of the ethanolic extract of *Andrographis paniculata* on glutathione-S-transferase enzyme in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



Values are presented as mean ± standard deviation.

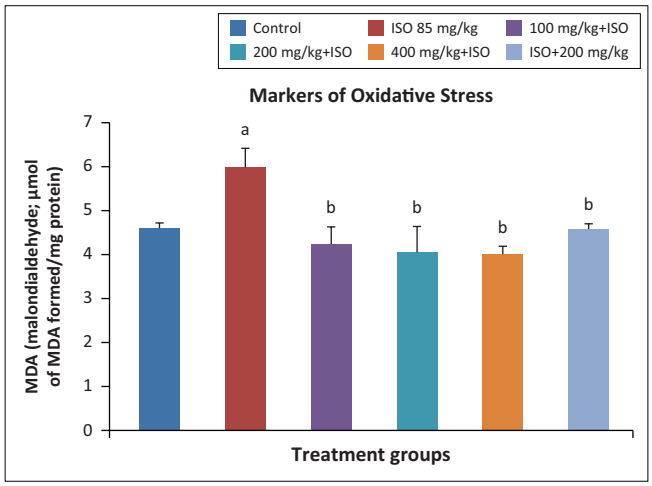
a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 7: Effect of the ethanolic extract of *Andrographis paniculata* on reduced glutathione in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$). The effect of *A. paniculata* on reduced glutathione (GSH).

thiols and non protein thiol but a significant decrease in the level of myeloperoxidase in all extract-treated groups except that of post-treatment phase (Figures 10-12). Although no significant histopathological changes were recorded for the extract, the ISO group had marked histopathological changes (Figure 13). Immunohistochemistry showed that ISO caused higher expressions of cardiac CRP and cTn1 and decrease in IL-10β but vice versa for the extract (Figures 14–16).

Discussion

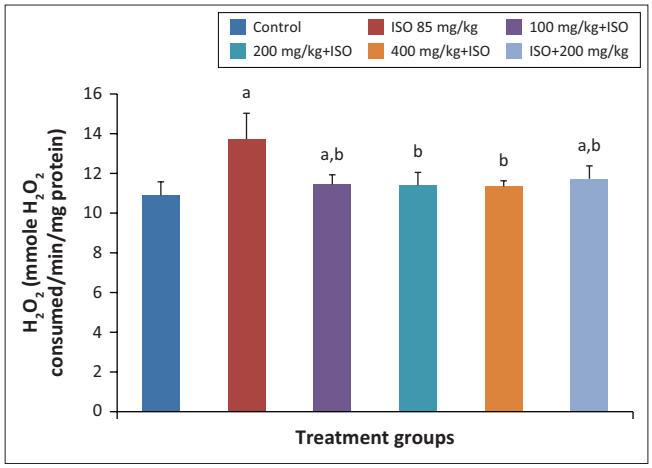
Andrographis paniculata has long been used as in traditional medicine for the treatment and prevention of various ailments. The active constituent of *A. paniculata* is andrographolide; its medicinal properties have been reported (Tan et al. 2016).



Values are presented as mean ± standard deviation.

a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

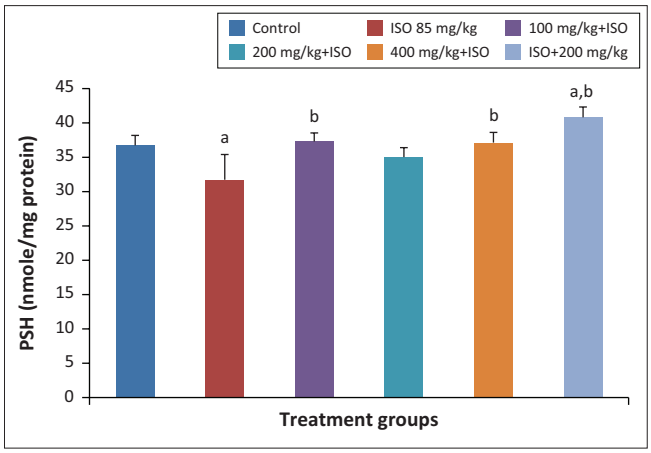
FIGURE 8: Effect of the ethanolic extract of *Andrographis paniculata* on lipid peroxidation in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



Values are presented as mean ± standard deviation.

a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

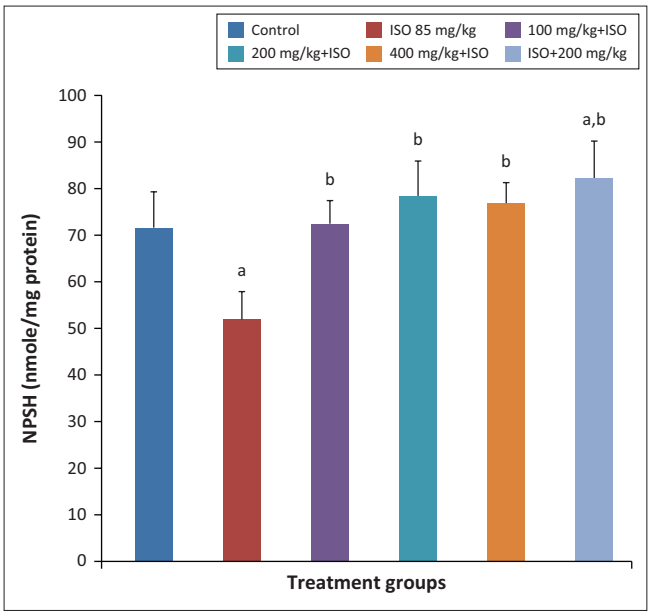
FIGURE 9: Effect of the ethanolic extract of *Andrographis paniculata* on hydrogen peroxide in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



The results showed the effect of ethanol extract of *Andrographis paniculata* (EEAP) on protein thiol (PT) generation where values are presented as mean ± standard deviation.

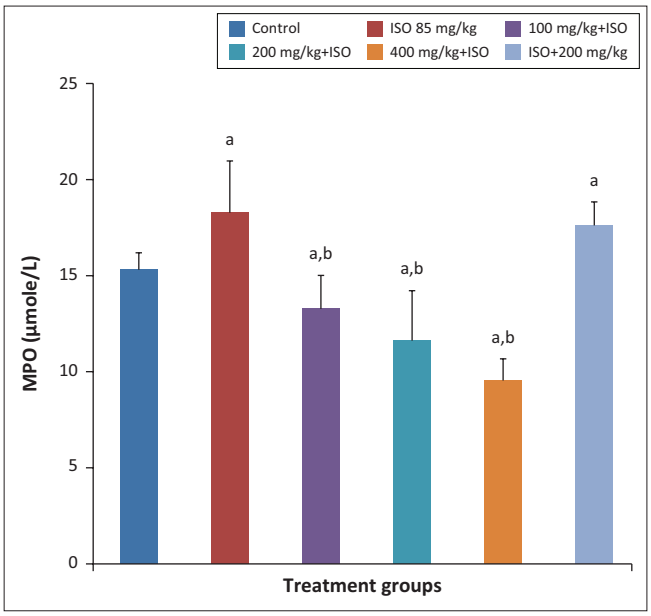
a, indicates significant difference ($p < 0.05$) when compared with control (Grp A); b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 10: Effect of the ethanolic extract of *Andrographis paniculata* on protein thiol in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



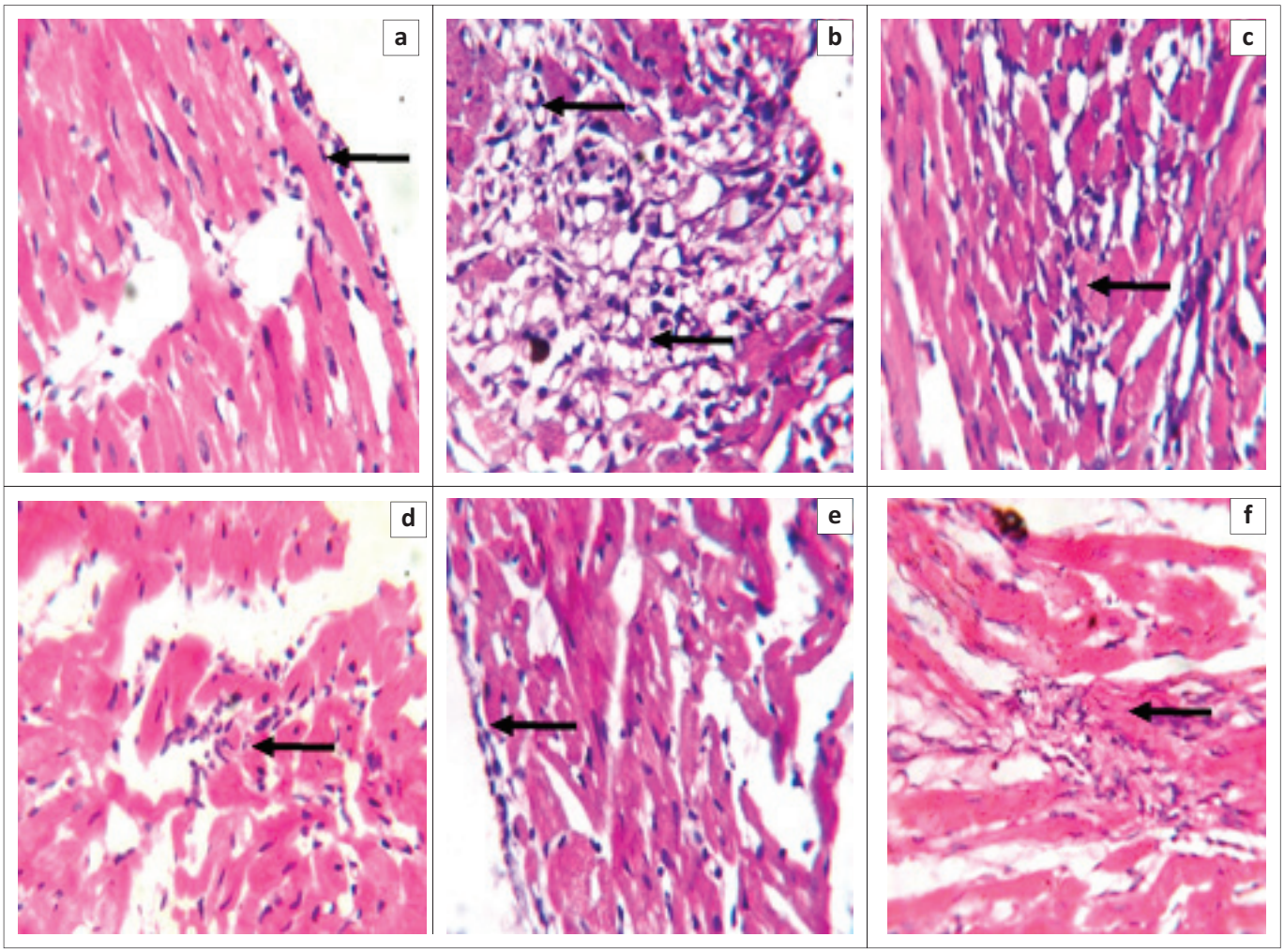
Values are presented as mean ± standard deviation.
a, indicates significant difference ($p < 0.05$) when compared with control (Grp A);
b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 11: Effect of the ethanolic extract of *Andrographis paniculata* on lipid peroxidation in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



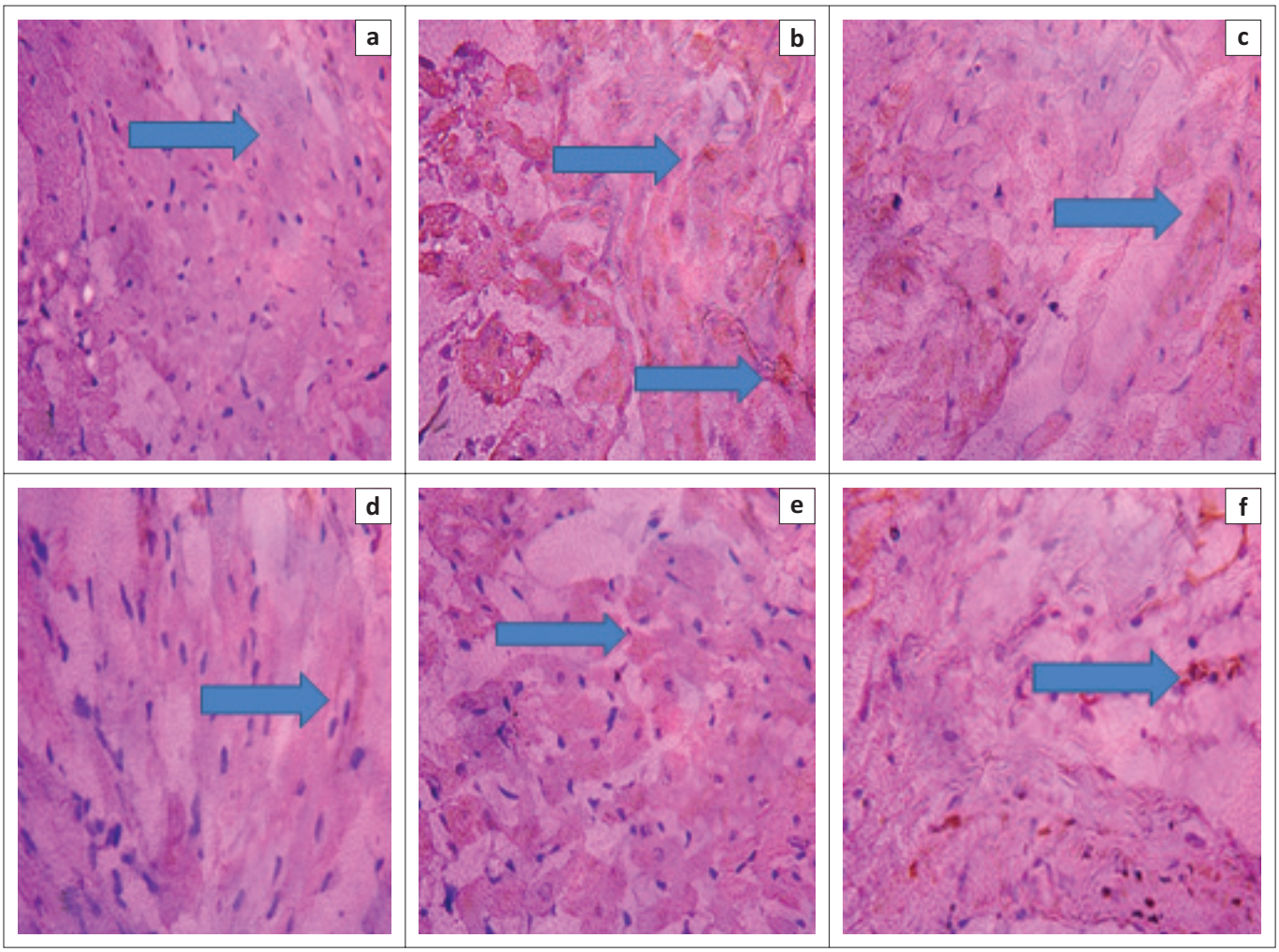
Values are presented as mean ± standard deviation.
a, indicates significant difference ($p < 0.05$) when compared with control (Grp A);
b, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B).

FIGURE 12: Effect of the ethanolic extract of *Andrographis paniculata* on myeloperoxidase in isoproterenol-induced myocardial infarction using rats as a model ($n = 5$).



(a) Control – shows no visible lesion. (b) ISO – shows severe infiltration of inflammatory cells. (c) 100 mg/kg + ISO and (d) 200 mg/kg + ISO – show mild infiltration of inflammatory cells. (e) 400 mg/kg + ISO – shows no visible lesion. (f) ISO + 200 mg/kg – shows mild infiltration of inflammatory cells. Mag. ×400

FIGURE 13: The histopathology of heart from isoproterenol-induced myocardial infarction using rats as a model.



(a) Control – shows positive and low expression of CTnI. (b) ISO – shows higher expression of CTnI than control. (c) 100 mg/kg + ISO, (d) 200 mg/kg + ISO, (e) 400 mg/kg + ISO and (f) ISO + 200 mg/kg – show lower expression of cTn than (b). The slides were counterstained with high-definition haematoxylin. Mag. x100.

FIGURE 14: Immunohistochemistry of cardiac troponin in heart of isoproterenol-induced myocardial infarction rats.

TABLE 2: Effects of the ethanolic leaf extract of *Andrographis paniculata* on ALT, AST and nitric oxide in isoproterenol-induced myocardial infarction in rats.

	Groupings		Pre-treated groups			Post-treated group
Parameters	**Control**	**ISO**	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	**200 mg/kg**
ALT (U/L)	14.51 ± 0.02	14.67 ± 0.05*	14.45 ± 0.04*,**	14.48 ± 0.04**	14.45 ± 0.01**	14.40 ± 0.01*,**
AST (U/L)	19.91 ± 0.01	19.97 ± 0.02*	19.88 ± 0.02**	19.88 ± 0.01*,**	19.88 ± 0.02*,**	19.87 ± 0.02*,**
NO (µmole/mg protein)	4.11 ± 0.68	1.72 ± 0.47*	4.16 ± 0.27**	3.41 ± 0.19**	2.91 ± 0.56*,**	3.07 ± 0.34*,**

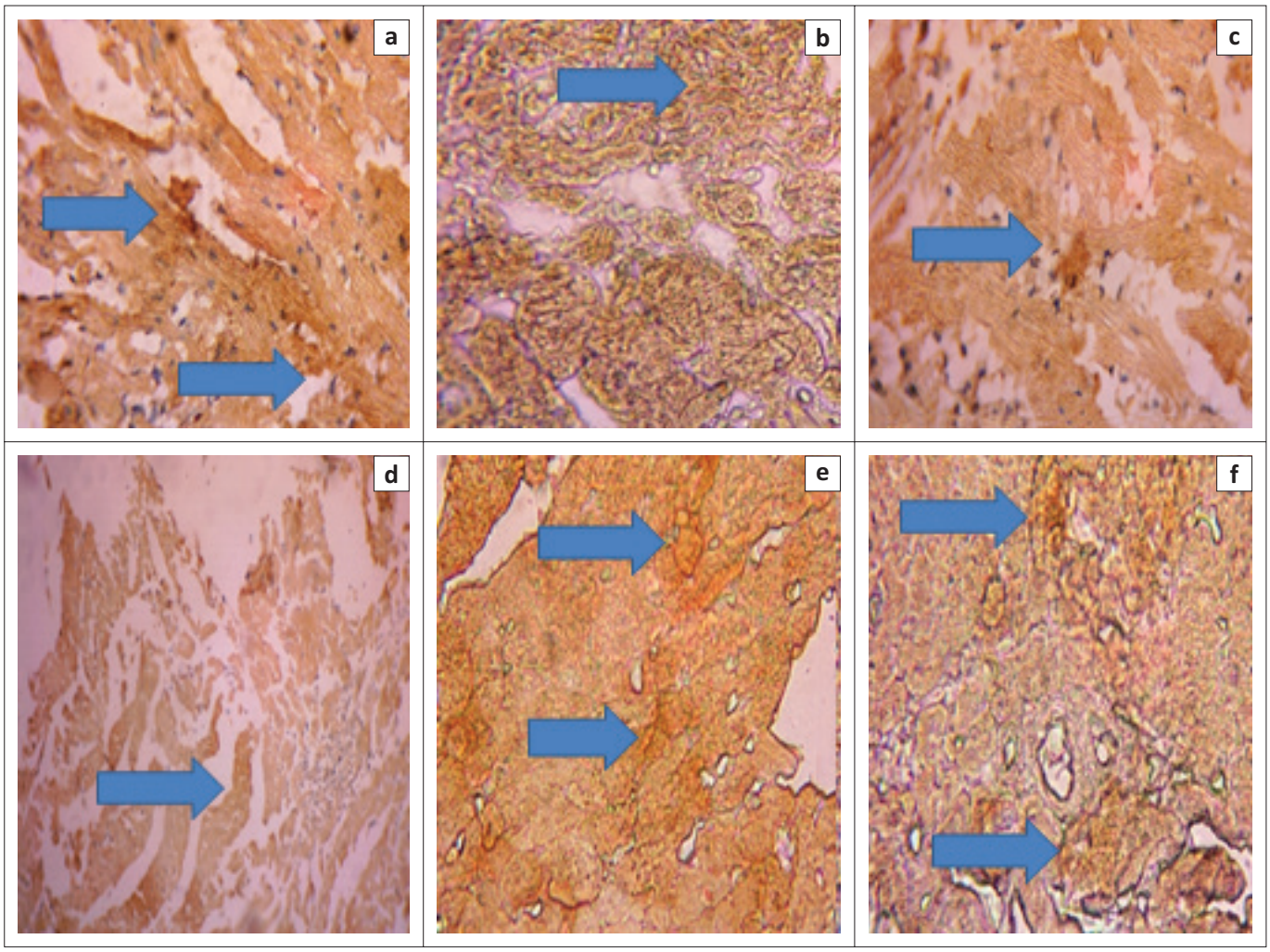
Note: Values are presented as mean ± standard deviation.

*, indicates significant difference ($p < 0.05$) when compared with control (Grp A); **, indicates significant difference ($p < 0.05$) when compared with ISO treated only (Grp B)

The proverbial mechanisms of ISO-induced MI include lipid metabolism disorders, large excess generation of ROS, oxidative stress as well as increased intracellular Ca^{2+} overload (Al-Yahya et al. 2013). Adrenochrome and other oxidation metabolites of catecholamines can cause cell necrosis and contractile failure in the rat's heart (Liaudet, Calderari & Pacher 2014). In this study, ISO caused MI via the abnormalities in the generation of free radical and inflammation. This study was designed to evaluate the cardioprotective effects of EEAP on isoproterenol-induced MI in rats.

To understand the protective effect of EEAP on cardiac abnormalities, hemodynamic parameters were included into the experimental study. Isoproterenol caused an intensive myocardial necrosis along with a profound decrease in systolic blood pressure, diastolic blood pressure and mean arterial pressure. The significant decrease in the levels of systolic, diastolic and mean arterial pressure may lead to coronary hypotension as seen in this study. In a study by Owens and O'Brien (1999), it was concluded that in patients suffering from ischemic heart disease and hypotension, symptomatic and silent ischaemia occurred in a temporally causal relation with hypotension, particularly for diastolic pressures. It thus suggests that patients with coronary disease may be susceptible to ischemic events that could be incurred as a result of low blood pressure. This study demonstrated that EEAP at all doses significantly prevented isoproterenol-induced cardiac abnormalities.

The results of haematological analysis in this study showed that ISO caused significant increase in the levels of



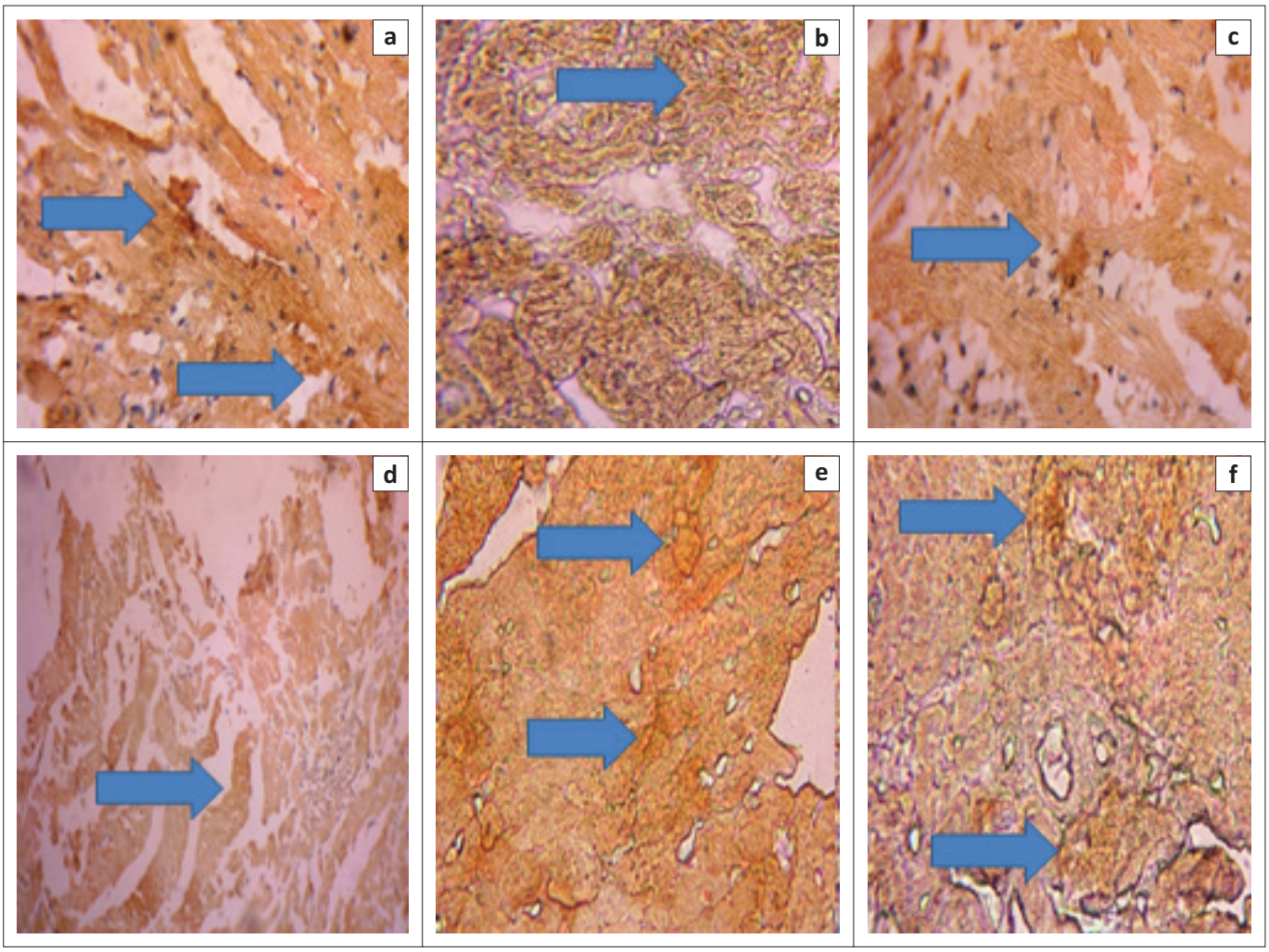
(a) Control – shows positive and low expression of CRP. (b) ISO – shows higher expression of CRP than control. (c) 100 mg/kg + ISO, (d) 200 mg/kg + ISO, (e) 400 mg/kg + ISO and (f) ISO + 200 mg/kg – show lower expression of CRP than (b). The slides were counterstained with high-definition haematoxylin. Mag. x100.

FIGURE 15: Immunohistochemistry of C-reactive protein in heart of isoproterenol-induced myocardial infarction rats.

white blood cells (WBC) and packed cell volume (PCV). The increase in the level of WBC could be explained in terms of necrosis caused by this agent leading to WBC mobilisation (Khalil et al. 2015). Ethanol extract of *A. paniculata* caused a significant increase in WBC, and this may be because of immunostimulant effect of the extract. This is in accordance with an earlier study by Adedapo, Obadan and Ohore (2009), where it was shown that an 800 mg/kg dose of the aqueous extract of *A. paniculata* caused a significant increase in the levels of WBC. Handa and Sharma (1990) in their study also observed an increase in the level of WBC. Puri et al. (1993) were of the opinion that the increase in the levels of the WBC differentials may be because of the presence of andrographolide, which is said to possess immunostimulatory properties.

Use of high concentration of ISO had been reported to induce severe oxidative stress and result in necrotic lesions in the myocardium of rats (Rona 1959; Yerra et al. 2006). The increased generation of ROS and/or depletion of the antioxidants in the defence system may contribute to oxidative stress and affect the pathogenesis of MI (Sawyer et al. 2002). Free radical scavenging enzymes such as SOD and GPx (Sawyer 2002) are the first-line cellular defence against oxidative stress, eliminating reactive oxygen radicals such as superoxide anion and hydrogen peroxide, and preventing the formation of more reactive radical of hydroxyl radical. In this study, rats pre-treated and post-treated with EEAP showed increased activities of these enzymes, which strongly suggested that EEAP has the ability to check the deleterious effects of free radicals in ISO-induced rats in agreement with the previous report by Sivakumar and Rajeshkumar (2015) which indicated that *A. paniculata* enhanced antioxidant enzymes.

GSH, protein thiol and non-protein thiol are non-enzymatic defences, which are important for maintaining cell integrity because of its reducing properties (Hadi et al. 2016). The function of GSH and thiols is to serve as the reductant of toxic peroxides and it also helps in keeping the enzymes in an active state by preventing the conversion of sulfhydryl group to disulphide group. In addition, GSH is a co-substrate for the detoxification of peroxides by GPx and of toxic metabolites by GSTs (Halliwell & Gutteridge 1989). When there is deficiency or depletion of this enzyme, it causes damage to the macromolecules or membranes. The deficiency of GSH



(a) Control – shows positive and higher expression of IL-10. (b) ISO – shows lower expression of IL-10 than control. (c) 100 mg/kg + ISO, (d) 200 mg/kg + ISO, (e) 400 mg/kg + ISO and (f) ISO + 200 mg/kg – all show higher expression of IL-10 than (b). The slides were counterstained with high-definition haematoxylin. Mag. x100.

FIGURE 16: Immunohistochemistry of interleukin-10 in heart of isoproterenol-induced myocardial infarction rats.

caused by ISO indicates its interaction with biomembrane and subsequent peroxidising action. The inhibitory effect on depletion of GSH levels by pre-treatment and post-treatment with EEAP in rats further revealed the protective effect of this extract on isoproterenol-induced MI, and this effect may be because of the antioxidant property of EEAP. Hassan et al. (2017) have reported this and is thus a confirmation of the antioxidant effect of this extract.

Lipid peroxidation, an essential pathogenic event in myocardial necrosis, is a sensitive marker of oxidative stress induced by ISO. Increased level of MDA, one of the lipid peroxidation end products, reflects the intensity of damage of cardiac constituents (Khalil et al. 2015). Malondialdehyde level is commonly used as a marker of oxidative stress (Maddock & Pariante 2001). In this study, there was an increase in MDA level in the ISO treated group, and previous studies have reported that ISO-induced MI could be as a result of induction of free radical-mediated lipid peroxidation, because of stress condition in rats (Zhou et al. 2016). The results presented in this study indicated that EEAP could decrease ISO-induced MDA content elevation. The decreased level of MDA in heart tissues might be because of the enhanced activities in antioxidant enzymes like SOD and GPx. It is quite possible that the free radicals induced by ISO were effectively neutralised and/or scavenged, resulting in the cardioprotective effect of EEAP.

After the onset of damage to myocytes as a result of deficient oxygen or glucose supply and oxidative stress caused by ISO, the cardiac membranes become permeable or even rupture, which results in the leakage of enzymes (Selaraj & Pugalendi 2012). Consequently, the myocardial enzymogram in serum changes constitutes an index to ascertain the alterations of membrane integrity and degree of myocardial injury caused by oxidative stress (Wang et al. 2016). Myeloperoxidase (MPO) is abundant in the granules of inflammatory cells, and it is an important enzyme in the generation of ROS by conversion of hydrogen peroxide to species including • OH, ONOO-, hypochlorous acid (HOCl) and NO_2 (Furtmuller et al. 2003; Zederbauer et al. 2007). Hydrogen peroxide (H_2O_2), an ROS, has been suggested as a mediator of vascular structural and functional alterations observed in hypertension (Alvarez et al. 2007). This study revealed that ISO caused high MPO activity, indicative of necrosis-induced inflammation of cardiac tissue. Treatment with EEAP at both

pre-treatment and post-treatment phases was found to significantly lower ISO-induced elevation in the activity of MPO enzyme. This result is in agreement with the work of Adedapo et al. (2015) who reported anti-inflammatory properties of *A. paniculata*.

Once the cardiomyocytes were injured because of ISO administration, this would be followed by disruption of associated cell membranes, and these intracellular proteins were then released into the circulation, promptly increasing the serum levels of these enzymes during the acute phase of necrosis. Although AST and ALT are not specific enzymes for MI because of their wide distribution in the body, they can be used as an early predictor of tissue damage (Dianita et al. 2015). In this study, we observed an increase in the serum activities of AST and ALT in rats treated with ISO. The release of cellular enzymes reflects the alterations in plasma membrane integrity and/or permeability as a response to α-adrenergic stimulation. This might be because of the damage caused to the sarcolemma by the α-agonist that has rendered it leaky. Isoproterenol induction produces free radicals via adrenoceptor mechanism and affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis (Upaganlawar et al. 2011). Ethanol extract of *A. paniculata* decreased the activities of these enzymes in serum. This could be because of the protective effect of EEAP on the myocardium, thus reducing the cardiac damage thereby restricting the leakage of these enzymes.

Furthermore, to elucidate the possible mechanism of EEAP on ISO-induced MI, serum NO was evaluated. Nitric Oxide may play a critical part in cardiac injury and hypertrophy (Yang et al. 2011). For instance within the vasculature, NO induces vasodilation, inhibits platelet aggregation, prevents neutrophil or platelet adhesion to endothelial cells, inhibits smooth muscle cell proliferation and migration, regulates programmed cell death (apoptosis) and maintains endothelial cell barrier function (Rosselli, Keller & Dubey 1998). NO is known to be deficient in chronic progressive renal disease (CRD) and in end-stage renal failure (ESRD) (Reyes, Karl & Klahr 1994), and this could result from arginine deficiency (Rosselli et al. 1998) which may be caused by a loss of functional renal mass, increased endogenous NO synthase (NOS) inhibitors that accumulate in renal failure (Vallance et al. 1992) and/or other causes, such as increased oxidant stress (Vaziri, Ovelisi & Ding 1998). Low NO production may also contribute to and/or exacerbate the progression of CRD by both hemodynamic and renal growth-promoting actions (Schmidt & Baylis 2000). This study demonstrated that serum NO level was increased in EEAP treated rats. Previous study in eNOS-over expressed mouse model had proved that released NO can attenuate ISO-induced hypertrophy (Ozaki et al. 2002).

Cardiac troponin T has been shown to be highly specific and a sensitive marker in the determination of myocardial cell injury. The studies by O'Brien et al. (1997) and Adamcova et al. (2016) have shown that cTnT is a powerful biomarker in laboratory animals for sensitive and specific detection of cardiac injury arising from various causes. In this study, there is an increase in the expression of CTnI in heart tissue of rats treated with ISO. This increase in troponin expression predicts the risk of both cardiac death and subsequent infarction. The observation of the increased expression of CTnI in heart tissue of rats treated with ISO is consistent with similar reports by Acikel et al. (2005) and Othman et al. (2017). In this study, treatment with EEAP decreased the expression of CTnI in heart tissue of ISO-induced MI in rats. Down regulation of cardiac troponin by EEAP showed the ability to protect against myocardial injury in rats. This could be because of the reduction of the degree of damage in the myocardium by the drug and EEAP.

Increased expression and plasma concentrations of inflammatory markers and mediators are seen in patients suffering from cardiovascular diseases (Blake & Ridker 2001; Sesso et al. 2003). C-reactive protein, in particular, has been demonstrated as an independent risk factor for the development of hypertension. Epidemiological studies have shown that plasma levels of high-sensitivity CRP (hsCRP) are a powerful predictor of ischemic cardiovascular events in patients with stable or unstable angina (Blake et al. 2003; Thorand et al. 2003). C-reactive protein has also been associated with increased risk of diabetes (Barzilay, Peterson & Cushman 2004). Several studies report that the level of CRP significantly increased in acute MI (Kim et al. 2016). CRP is a known marker of systemic inflammation, which has been linked with an increased risk of MI, and potentially vital device in primary prevention of CVD (Itrat et al. 2017). It was the first acute phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage (Rosselli et al. 1998). In this study, there is increased expression of CRP in ISO treated rats. In acute myocardial infarcts, CRP is co-deposited with activated complement (Lagrand et al. 1997), and research findings have shown that the CRP response did not only reflect tissue damage in this context but may significantly contribute to the severity of ischemic myocardial injury (Griselli et al. 1999). It is very clear that CRP plays a role in the pathogenesis of cardiovascular disease, and as a marker and predictor of cardiovascular disease, CRP possesses numerous cardiovascular effects including clotting, generation of oxygen radicals, increase in the expression of adhesion molecules and plasminogen activator inhibitor-1, and plaque destabilisation, and these could result in cardiovascular disease. The lowering of the level of CRP in this study by EEAP is a pointer to its ability to halt cardiovascular disease, hence the cardioprotective effect through its antioxidant and anti-inflammatory properties.

In addition to homeostasis of proinflammatory and anti-inflammatory effects, cytokines play a major role in cardiac pathophysiological conditions like MI (Ono et al. 1998). IL-10 is a Th_2-type cytokine that is produced by a wide range of immunological cell types, including monocytes or macrophages, and it is a potent inhibitor of the proinflammatory cytokines and chemokines (Akdis & Blaser 2001). Interestingly, in this study, there was an increase of IL-10 in heart tissues of rats both at pre-treatment

and post-treatment phases with EEAP suggesting its cardioprotective effect.

ISO-induced MI is also manifested by altered histopathological features including marked necrosis, severe infiltration of inflammatory cells and disorganisation of myocardium. This increase in the inflammatory cells may have been responsible for the increase in the levels of WBC noted in this study. In the study on the ISO-induced myocardial damage, it was discovered that the cardiac lesions varied with treatment duration and doses and that numerous macrophages were observed in the necrotic areas (Zhang et al. 2008). The normal control rats showed normal cardiac fibres without any infiltration of inflammatory cells, whereas the tissues from *EEAP treated rats* showed a near normal myocardium (Figure 13).

Conclusion

The results from this study indicated that the ethanol leaf extract of *A. paniculata* has a protective effect on isoproterenol-induced MI. Amongst the groups treated with ISO (85 mg/kg) and *EEAP*, the best results were achieved in the EEAP (ISO + 200 mg/kg) group. Thus, these findings indicate that EEAP has a cardioprotective effect against isoproterenol-induced MI in rats.

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Competing interests

The authors declare that they have no financial or personal relationships, which may have inappropriately influenced them in writing this article.

Authors' contributions

B.O.A., T.O.A., A.D.A., A.A.O. and T.O.O. performed the experiments. A.A.A. was the project leader and along with A.E.A. and M.A.Y. designed the experiment. A.A.A. drafted the manuscript and all authors read and approved the final draft.

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