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Enalapril Confers Protective Effect on Isoproterenol-Induced Myocardial Infarction in Rats through Downregulation of Cardiac Troponin, C-reactive Protein, Upregulation of IL-10β as Well as Anti-Oxidant and Anti-inflammatory Activities

B. O. Adeoye[1], T. O. Ajibade[2], E. R. Asenuga[3], O. A. Adejumobi[4], A. A. Oyagbemi[2], T. O. Omobowale[4] and A. A. Adedapo[1]*

[1]*Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Nigeria.*
[2]*Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria.*
[3]*Department of Veterinary Physiology and Pharmacology, University of Benin, Nigeria.*
[4]*Department of Veterinary Medicine, University of Ibadan, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors BOA, ERA and AAO carried out the experiment. Authors TOA, OAA and TOO measured the ECG and blood pressure while author AAA designed and drafted the final manuscript. All authors read and approved the final manuscript before its submission.

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ABSTRACT

Myocardial infarction is an irreversible death of heart muscle secondary due to prolonged lack of oxygen supply. The present study was designed to evaluate the protective effect of enalapril in isoproterenol-induced myocardial infarction in rats using changes in haemodynamic, biochemical, histopathological and immunohistochemistry parameters. Twenty-one male Wistar rats divided into

**Corresponding author: E-mail: adedapo2a@gmail.com, ae.adedapo@ui.edu.ng;*

three groups were used where the control (group A) was administered for normal saline which continued for 7 days, group B animals received normal saline for 7 days and thereafter isoproterenol (ISO) at 85 mg/kg on day 8 and 9. Group C animals were pretreated with enalapril (10 mg/kg) for 7 days and thereafter received ISO on day 8 and 9. On day 10, the blood pressure change in the animals were measured and thereafter sacrificed by cervical dislocation. The heart of each rat was removed, homogenized and used to assay for some oxidative stress markers and some antioxidant parameters. In this study, ISO caused myocardial infarction as seen by significant decrease in systolic, diastolic and mean arterial pressure but was corrected by enalapril. Enalapril caused significant increase in the levels of SOD, GPx, GST and GSH but significant decrease in MDA content and H_2O_2 generation. But reverse was the case for group B animals. Immunohistochemistry showed that ISO caused higher expressions of cardiac C-reactive protein (CRP) and cardiac troponins 1 (CTn1) and decrease in IL-10β but vice-versa for enalapril. No histopathological changes were recorded for enalapril. The study thus showed that enalapril significantly exhibits cardioprotective effects.

Keywords: Enalapril; myocardial infarction; cardioprotection; immunohistochemistry; antioxidant.

1. INTRODUCTION

Human health is being seriously threatened by cardiovascular diseases (CVD), which have been regarded as the main cause of death throughout the world [1-3]. Myocardial infarction (MI) is a common presentation of ischemic heart disease (IHD) and remains the major cause of death in the developed world. Though rapid advancements have been made in the treatment of coronary artery diseases (CAD), MI is still a major pathological issue worldwide [4]. Increased myocardial metabolic demand and decreased supply of oxygen as well as nutrients via the coronary circulation to the myocardium brings about myocardial infarction hence leading to cell injury. This pathological heart condition is one of the most lethal manifestations of cardiovascular diseases. Acute myocardial infarction or heart attack occurs when blood stops flowing to part of the heart leading to injury to the heart muscle due to the fact the heart is not receiving enough oxygen [5-9].

Isoproterenol [1-(3, 4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] (ISO) a synthetic catecholamine is a β-adrenergic agonist that is very important in the regulation of myocardial contractility and metabolism. It serves as a standard model for the study of potentially beneficial effects of numerous drugs on cardiac function [10,11]. ISO induces myocardial injury in rat because of the alteration in the physiological balance between production of free radicals and antioxidative defence system [12]. It thus causes the acute condition of myocardial necrosis, which can lead to cardiac dysfunctions, increased lipid peroxidation, altered activities of cardiac enzymes and antioxidants [13]. It has been observed that the pathophysiological and morphological changes observed in ISO-treated rats are similar to those observed in human MI [14].

Enalapril an Angiotensin-converting-enzyme inhibitor (ACE inhibitor) is a drug used primarily for the treatment of high blood pressure and congestive heart failure where it can be used alone or in combination with other antihypertensive agents. ACE inhibitors have also been found to be useful for other cardiovascular and kidney diseases including acute myocardial infarction, diabetic nephropathy, and cardiac failure [15]. The mechanism of action of ACE inhibitors involves reduction of the activity of the renin-angiotensin-aldosterone system (RAAS) [16].

In recent times, a novel strategy has been employed in drug discovery. It is the use of known and approved drugs and compounds for newer indications. This is termed drug repurposing. In this study, Isoproterenol was used to induce acute myocardial infarction and enalapril was then used to ameliorate this and then to see if it could serve as a repurposed drug for myocardial infarction.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Isopreterenol, enalapril, Tween 80, Biurett's 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) Greiss

reagent, phosphoric acid, sodium hydroxide, N 1-naphthyl ethylenediamine, sulphanilamide, distilled water, phosphate buffer saline, creatinine reagent, copper sulphate, tri chloro acetate, reduced glutathione (GSH), thiobarbituric Acid (TBA), trichloroacetic acid (TCA), ammonium ferrous sulphate, glacial acetic acid, potassium iodide, sorbitol, Ellman's reagent (DTNB), ethanol, urea reagent. All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK). All other chemicals, reagents and drugs used were of analytical grade.

2.2 Experimental Animals

All experiments and protocols described in present study were approved by the UI-ACUREC. Twenty one (21) male Wistar rats weighing between 90 to160 g were obtained from the Experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan for the experiment. They were allowed free access to standard rat pellets and fresh water *ad libitum*. The rats were housed in the animal house unit of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan with a 12 hour light duration. Pre-conditioning of the rats was done for two weeks before commencement of the experiment. The institutional approval was given to this study and the number is UI-ACUREC/App/2016/030

2.3 Cardioprotective Study

The animals were randomly divided into three (3) groups with seven (7) animals in each group, and the treatment was as follow: Animals in the control (group A) were administered normal saline, group B; isoproterenol at 85 mg/kg, while group C animals were pretreated with enalapril orally (10 mg/kg) for 7 days and thereafter administered ISO (85 mg/kg) subcutaneously on day 8 and 9. Blood pressure values of all the animals were carried out on day 10. At the end of the experimental period, blood samples were collected for haematology and 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 fifteen (15) minutes and processed for determination of serum myeloperoxidase, total protein, and xanthine oxidase, AST, ALT and nitric oxide. The heart of each rat was carefully removed and homogenized on ice and then used to assay for some oxidative stress markers and antioxidant parameters. Baseline cardiovascular parameters were obtained 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. The haemodynamic parameters assessed were; the systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) and were determined indirectly in nonanaesthesised rats, by tail plethysmography with the use of an electrosphygnomanometer (CODA, Kent Scientific, USA). The average of at least nine most consistent readings, taken in the quiescent state, following acclimatization, was recorded per animal.

Blood samples for serum chemistry were collected from the rats through retro-orbital vein after which the animals were sacrificed by cervical dislocation.

2.4 Preparation of Tissue Homogenate

The heart tissues of the rats were harvested on ice, rinsed with normal saline and homogenized in aqueous potassium buffer (0.1 M, pH 7.4) and the homogenate centrifuged at 12,000 rpm (4°C) for 15 min to obtain the supernatant fraction.

2.5 Determination of Biochemical Assay

Biuret method as described by Gornal et al. [17] was used to determine the protein concentrations of the various samples with a slight modification. To prevent precipitation of Cu^{2+} ions as cuprous oxide potassium iodide was added to the reagent. To determine the concentration of reduced glutathione the method of Beutler et al. [18] was used while glutathione peroxidase (GPX) activity was measured by the method of Rotruck et al. [19]. In this case, hydrogen peroxide was used as substrate to oxidize reduced glutathione to oxidized glutathione (GSSG). Estimation of Glutathione S-transferase (GST) was by the method of Habig et al. [20] 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 [21]. MDA content was measured in the heart as an index of lipid peroxidation [22]. Hydrogen peroxide generation was measured using Wolff's [23] method while the determination of Sulfhydryl (Thiol) content was by-the method of Ellman [24]. Nitric oxide was quantified as previously described [25].

2.6 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 that were about 5-6 µm thick were made and stained with haematoxylin and eosin for histopathological examination [26].

2.7 Immunohistochemistry of Cardiac Troponins-1, CRP and IL-10

The heart tissues obtained from the rats were paraffin embedded and then used for immunohistochemistry. Paraffin sections were melted at 60℃ in the oven but the dewaxing of the samples in xylene was followed by passage through ethanol of decreasing concentration (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 troponins 1, CRP antibody and I IL-10β (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 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 CRP, cardiac troponin and IL-10β intensive regions were viewed starting from low magnification on each slice then with 400 × magnifications using a photo microscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

2.8 Statistical Analysis

All values were expressed as mean ± standard deviation (SD). The test of significance between two groups was estimated by Student's t-test. One-way Analysis of Variance (ANOVA) with Tukey's post-hoc test using Graph pad prism 5.0 was also performed with p-values < 0.05 considered statistically significant.

3. RESULTS

In this study, ISO caused significant decreases in the levels of SBP, DBP and MAP while enalapril (ENA) caused significant increase though not to the same extent as the control (Figs. 1-3). The results of haematological analysis showed that ISO caused significant increases in the levels of WBC, PCV, MCV and MCH while ENA caused significant decrease in WBC and no changes relative to ISO (Table 1). ISO also caused significant increases in the levels of AST and ALT while ENA caused significant decreases in the levels of these enzymes. On the other hand, while ISO caused significant decrease in the level of NO, ENA caused significant increase (Table 2). ISO caused significant increases in the levels of oxidative markers such as MDA, H_2O_2 and MPO while ENA caused significant decreases in the levels of these markers in a similar fashion to the control (Figs. 4-6). Again, while ISO caused significant decrease in the levels of protein thiols and non-protein thiols, ENA caused a significant increase in the levels of these molecules (Figs. 7 and 8). The result also showed that ISO caused significant decrease in the levels of anti-oxidant markers such as SOD, GPx, GST and GSH but reverse is the case for ENA (Figs. 9-12). Histopathological examinations showed that while there is severe infiltration of inflammatory cells into the cardiac tissue, there was no visible lesion seen in the ENA and control groups (Fig. 13). The Immunohistochemical analysis showed that there were high expressions of cardiac troponin and CRP in ISO group but lower expression of these proteins in ENA and control group (Figs. 14 and 15). In the case of IL-10β, there was low expression of this protein in ISO group but higher expression in ENA and control group (Fig. 16).

4. DISCUSSION

Myocardial infarction (MI), one of the main causes of death from cardiovascular disease is defined as an acute condition of necrosis of the myocardium and it occurs as a result of imbalance between coronary blood supply and myocardial demand [27]. MI is known to cause local inflammation and apoptosis and this can result in cardiomyocyte damage [28]. ISO induces cardiac necrosis by several mechanisms, including increased oxygen consumption, poor oxygen utilization, increased calcium overload and accumulation, altered myocardial cell metabolism, increased myocardial cAMP levels, deranged electrolyte

milieu, altered membrane permeability, intracellular acidosis, and increased levels of lipid peroxides [11]. The pathophysiological changes that occurred in heart following isoproterenol administration in rats are comparable to those taking place in human myocardial infarction [29].

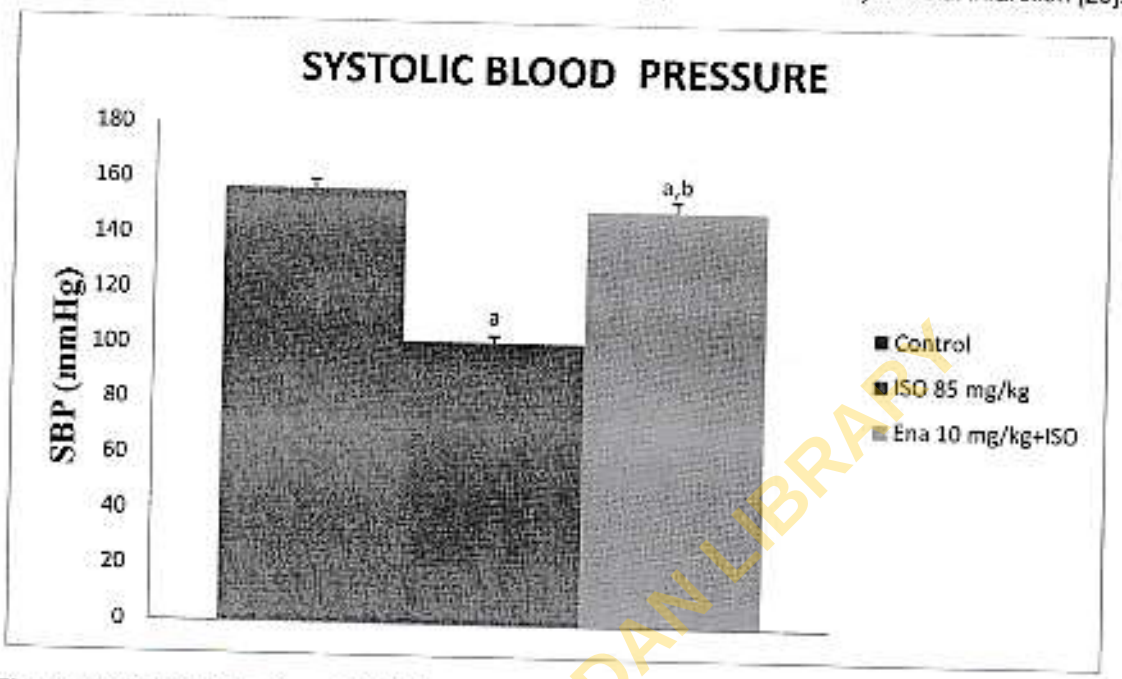

Fig. 1. Effect of enalapril on SBP in isoproterenol induced myocardial infarction using rats as a model. The superscript 'a' showed that ISO caused significant decrease when compared to control while superscript 'b' showed significant decrease when compared with ENA (n=7)

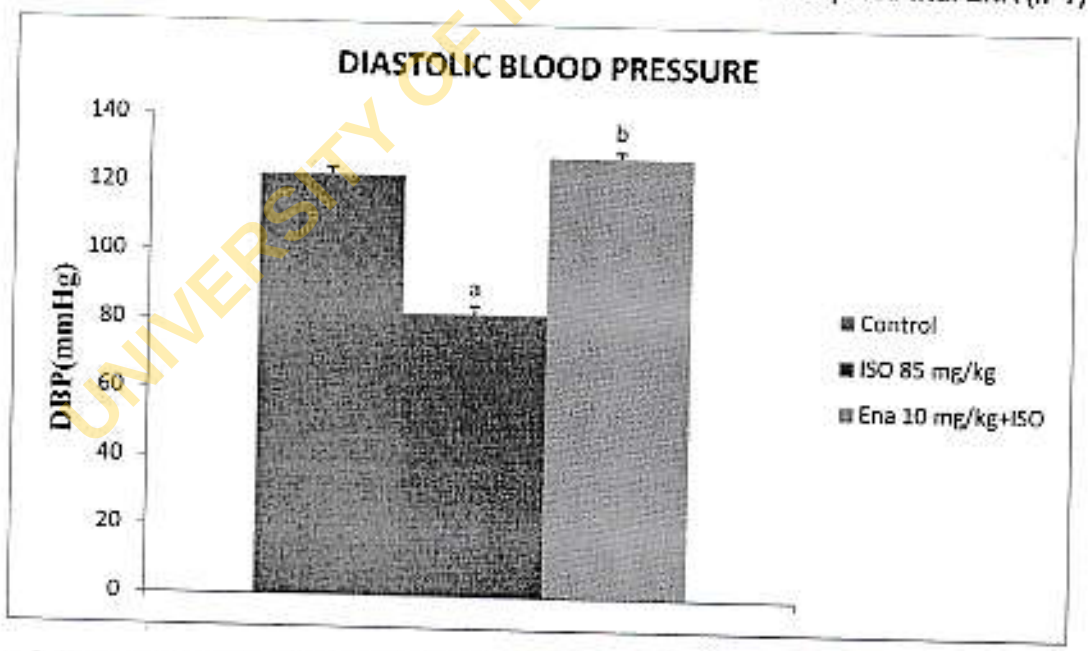

Fig. 2. Effect of enalapril on DBP in isoproterenol induced myocardial infarction using rats as a model. The superscript 'a' showed that ISO caused significant decrease in the level of this parameter compared to control while 'b' showed that ENA caused significant increase relative to control and ISO groups (n=7)

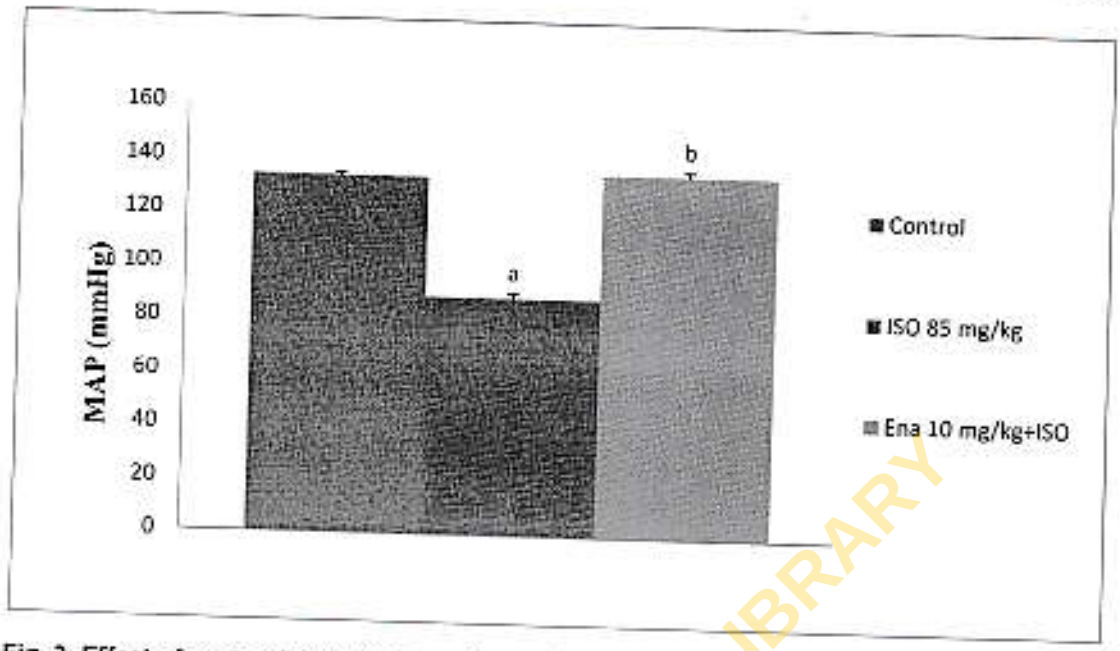

Fig. 3. Effect of enalapril MAP in isoproterenol-induced myocardial infarction using rats as a model. The superscripts showed that ISO caused significant decrease relative to ENA and control groups (n=7)

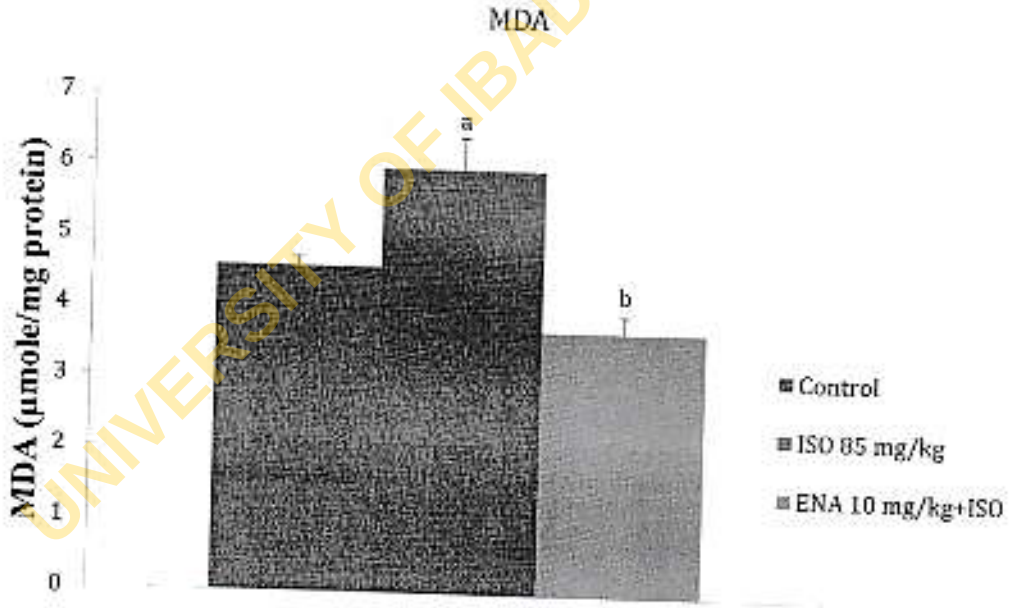

Fig. 4. Effect of enalapril on lipid peroxidation in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO). The superscript (a) showed that ISO caused significant decrease in the level of this parameter compared to control while (b) showed that ENA caused significant increase relative to control and ISO groups

Table 1. Effects of enalapril on RBC, WBC, HB, PCV, MCV, MCH and MCHC in isoproterenol-induced myocardial infarction using rats as a model (n = 7)

Parameters	Control	ISO	Enalapril
RBC (×1012/L)	4.75±0.90	4.96±0.43	5.03±0.69
WBC (103/µL)	5.47±0.38	6.71±1.13[a]	4.68±1.68[b]
HB (g/dl)	13.33±1.40	15.15±1.84	14.95±1.62
PCV (%)	45.75±4.65	54.25±4.25[a]	50.25±3.10
MCV (fl)	83.88±9.03	127.33±30.12[a]	98.87±22.76
MCH (pg)	26.41±3.48	38.64±8.08[a]	26.05±2.25
MCHC (%)	29.97±2.05	27.41±2.38	30.79±2.37

Values are mean ± SD, n =5, [a] - p < 0.05 compared with control, [b] - p < 0.05 compared with ISO. The superscript (a) showed that ISO caused significant decrease in the level of this parameter compared to control while (b) showed that ENA caused significant increase relative to control and ISO groups

Table 2. Effects of enalapril on ALT, AST and NO in isoproterenol-induced myocardial infarction using rats as a model (n=7)

Parameters	Control	ISO	Enalapril
ALT	14.51±0.02	14.67±0.05[a]	14.41±0.05[ab]
AST	19.91±0.01	19.97±0.02[a]	19.87±0.02[ab]
NO	4.11±0.68	1.72±0.47[a]	2.67±0.71[ab]

Values are mean ± SD, n =5, [a] - p< 0.05 compared with control, [ab] - p< 0.05 compared with ISO. The superscript 'a' showed that ISO caused significant decrease in the level of this parameter compared to control while 'b' showed that ENA caused significant increase relative to control and ISO groups

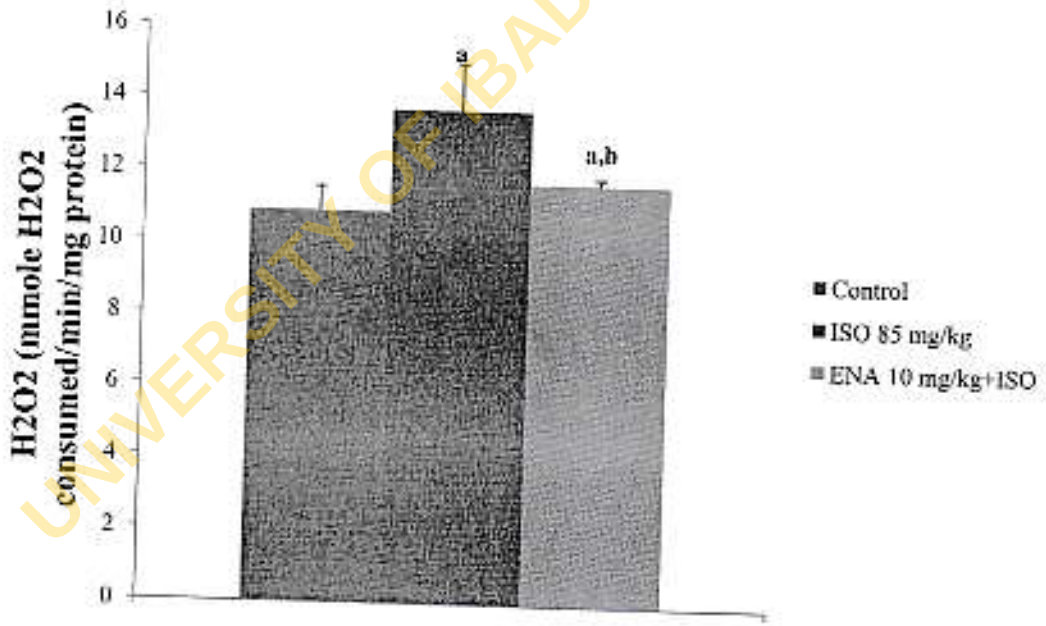

Fig. 5. Effect of enalapril on hydrogen peroxide generation in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference (p< 0.05) when compared with control (Grp A), whereas superscript 'b' indicates significant difference (p < 0.05) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

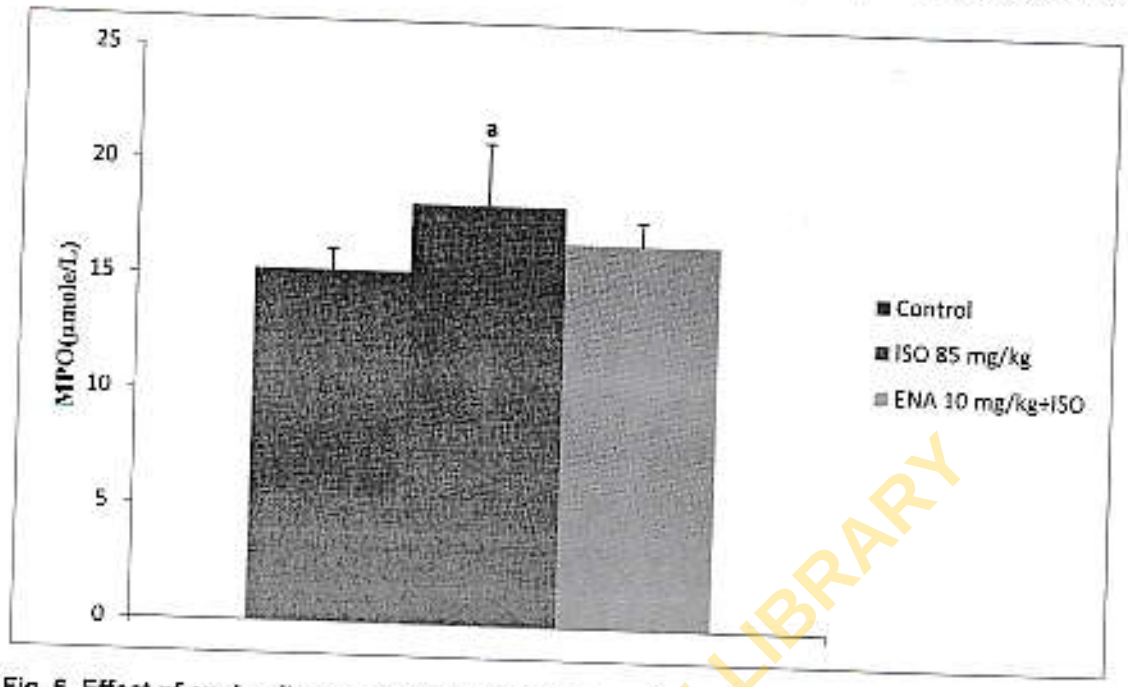

Fig. 6. Effect of enalapril on myeloperoxidase in isoproterenol-induced myocardial infarction using rats as a model (n=5). The superscript 'a' showed that ISO caused significant increase in the level of this parameter when compared to the control and ENA groups

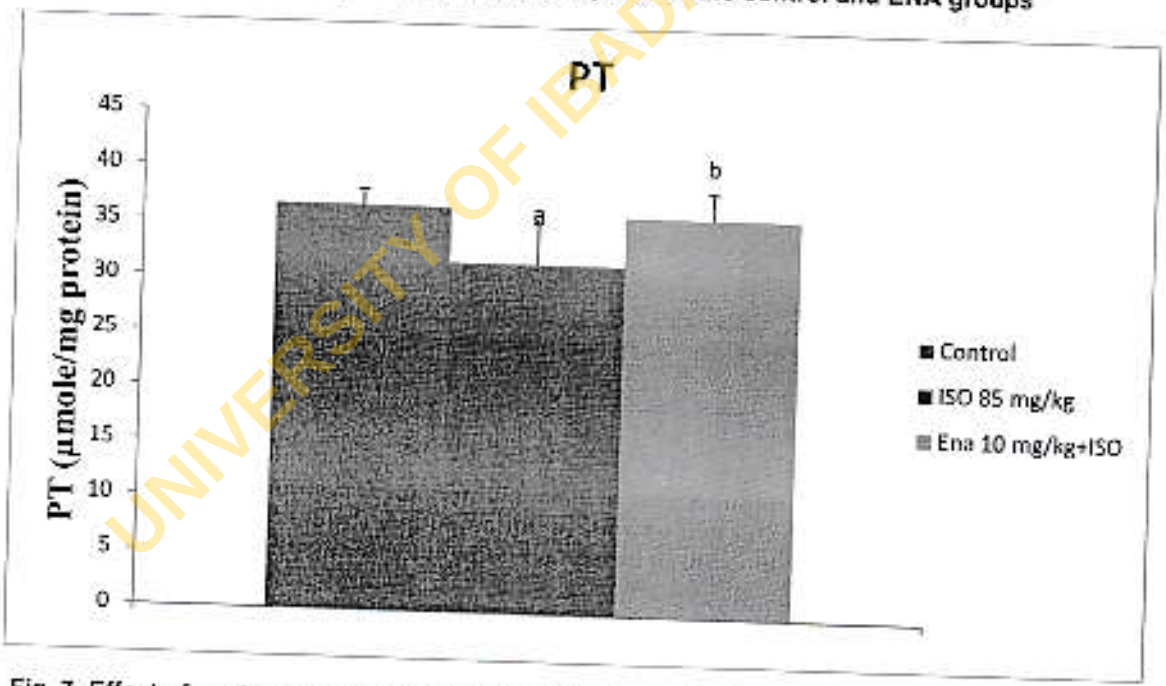

Fig. 7. Effect of enalapril on protein thiol in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference ($p<0.05$) when compared with control (Grp A), whereas superscript 'b' indicates significant difference ($p<0.05$) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

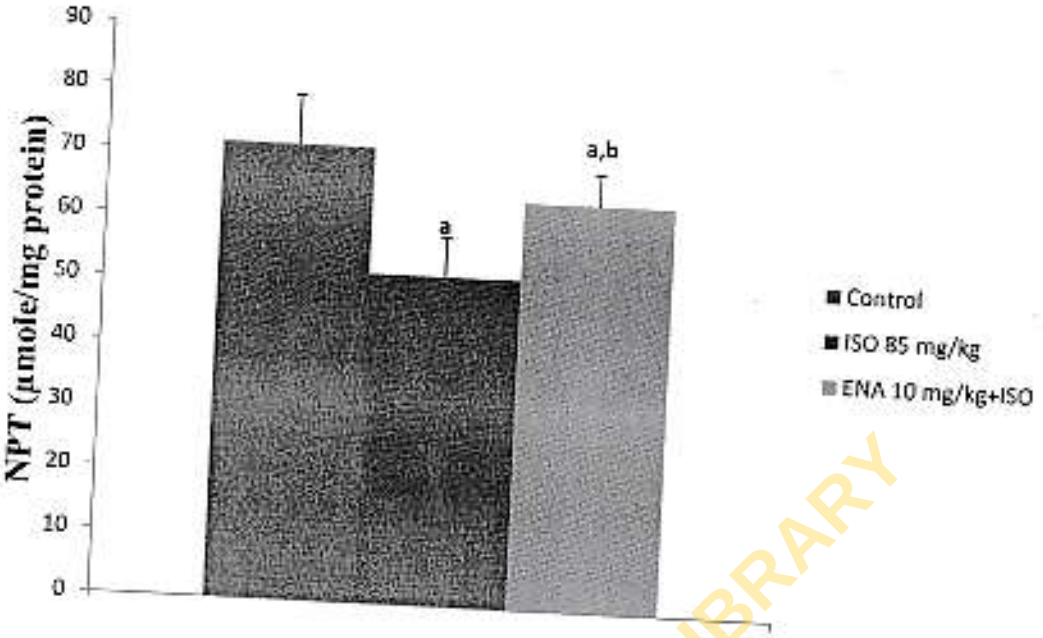

Fig. 8. Effect of enalapril on non-protein thiol in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference ($p<0.05$) when compared with control (Grp A), whereas superscript 'b' indicates significant difference ($p<0.05$) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

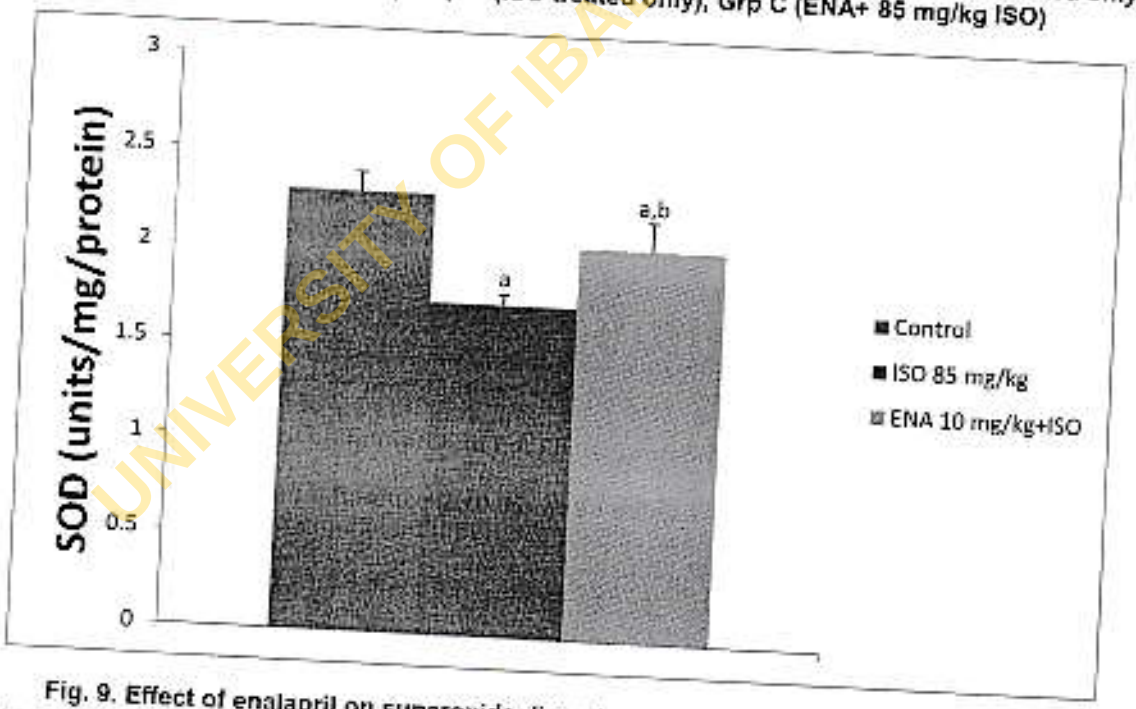

Fig. 9. Effect of enalapril on superoxide dismutase enzyme in isoproterenol-induced myocardial infarction (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference ($p<0.05$) when compared with control (Grp A), whereas superscript 'b' indicates significant difference ($p<0.05$) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

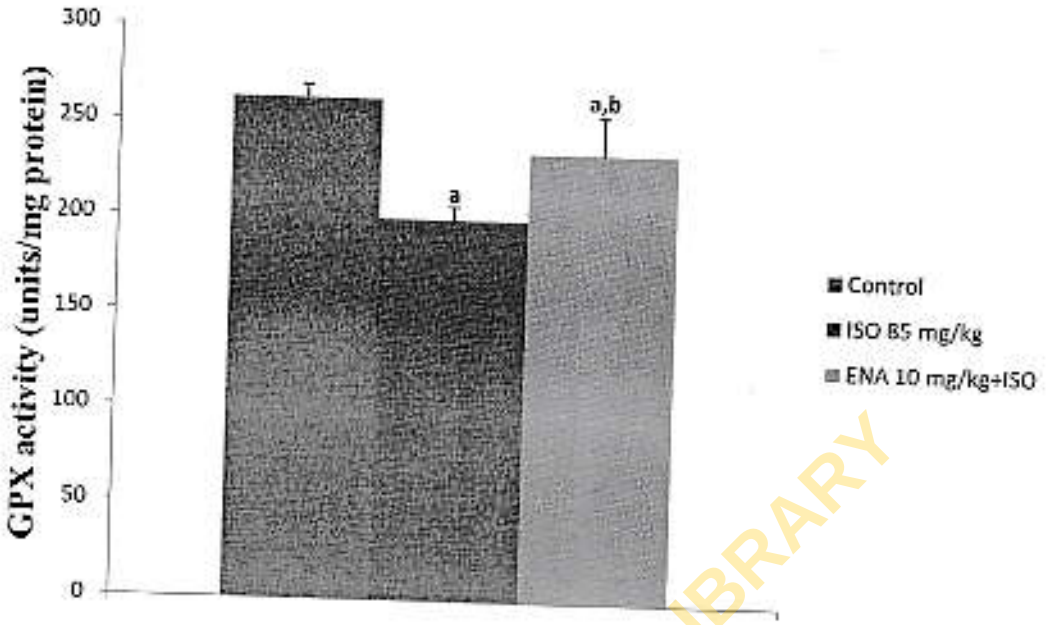

Fig. 10. Effect of enalapril on glutathione peroxidase enzyme in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference ($p < 0.05$) when compared with control (Grp A), whereas superscript 'b' indicates significant difference ($p<0.05$) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

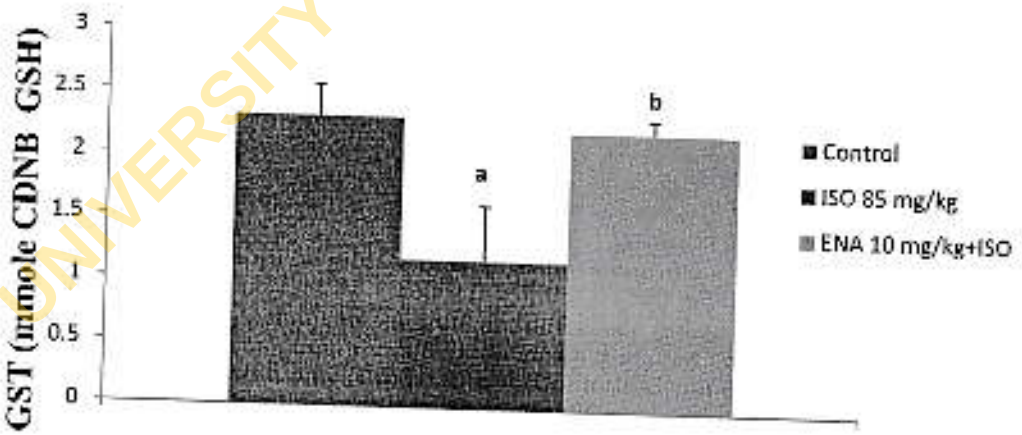

Fig. 11. Effect of enalapril on glutathione-s- transferase enzyme in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference ($p<0.05$) when compared with control (Grp A), whereas superscript 'b' indicates significant difference ($p<0.05$) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

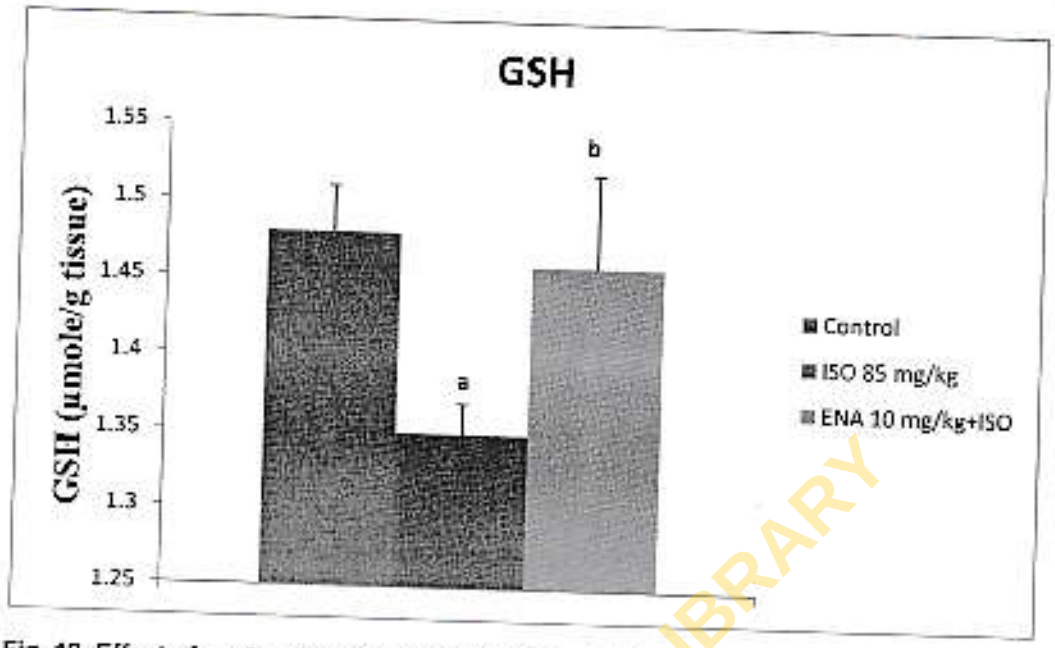

Fig. 12. Effect of enalapril on reduced glutathione in isoproterenol-induced myocardial infarction using rats as a model (n=5). Values are presented as mean ± standard deviation. Superscript 'a' indicates significant difference ($p<0.05$) when compared with control (Grp A), whereas superscript 'b' indicates significant difference ($p<0.05$) when compared with ISO treated only (Grp B). Grp A (Control), Grp B (ISO treated only), Grp C (ENA+ 85 mg/kg ISO)

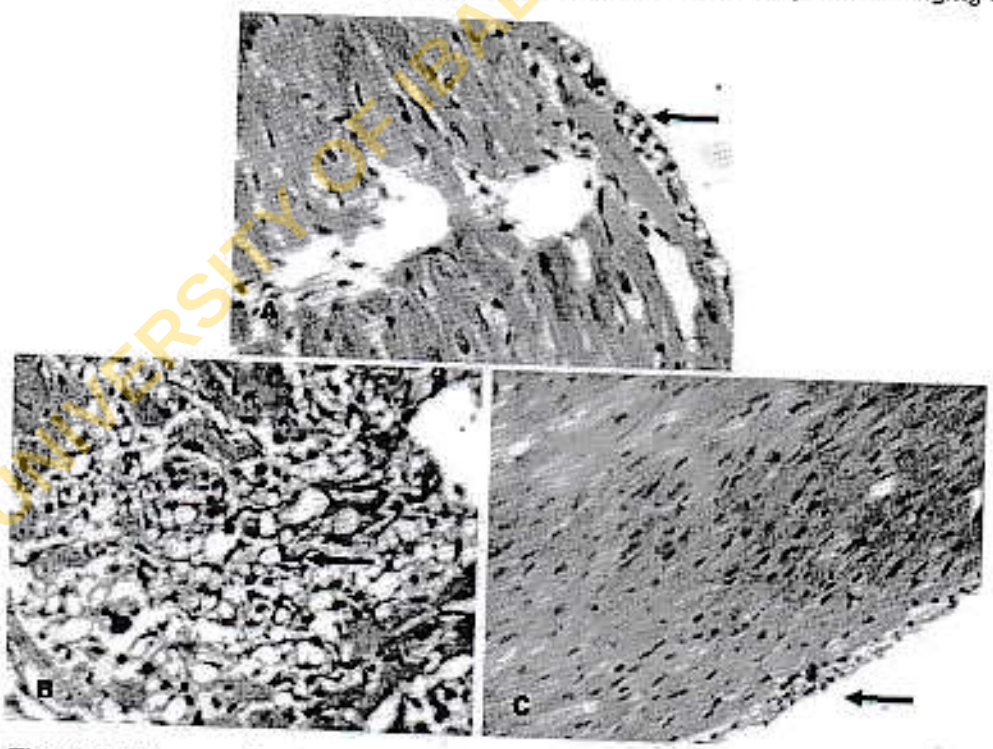

Fig. 13. The photomicrograph of heart from isoproterenol-induced myocardial infarction using rats as a model. A (Control) shows no visible lesion. B (ISO): shows severe infiltration of inflammatory cells. C (enalapril) shows no visible lesion. The slides were with H & E. Mag. ×400

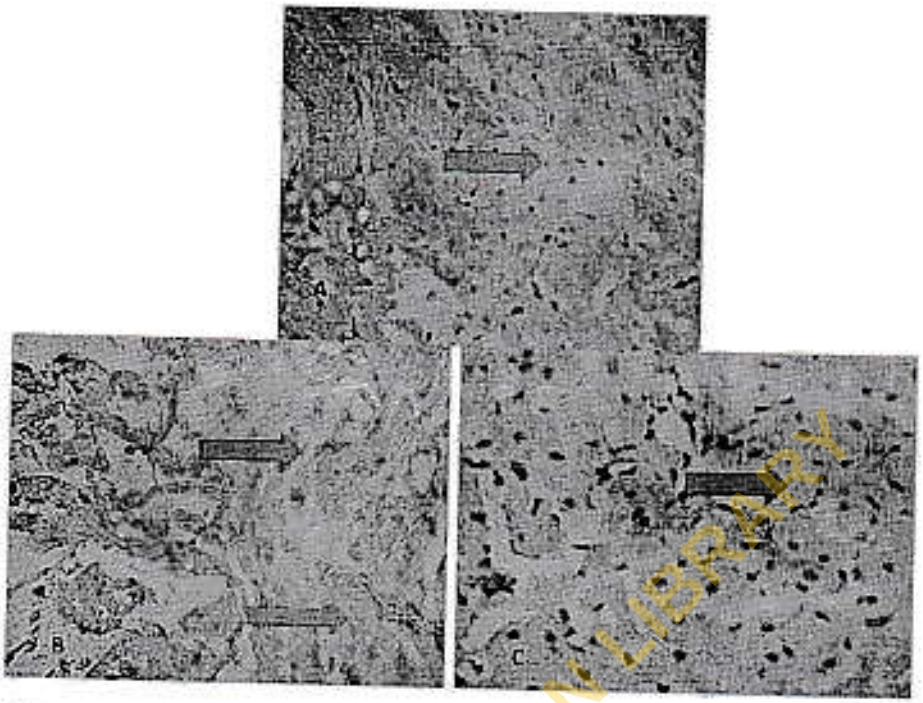

Fig. 14. Immunohistochemistry of cardiac troponin in heart of isoproterenol induced myocardial infarction rats. A (Control): show positive and low expression of CTnI, B (ISO): shows higher expression of CTnI than control, C (enalapril) shows lower expression of CTnI than B (ISO). The slides were counterstained with high definition haematoxylin. Mag. x100

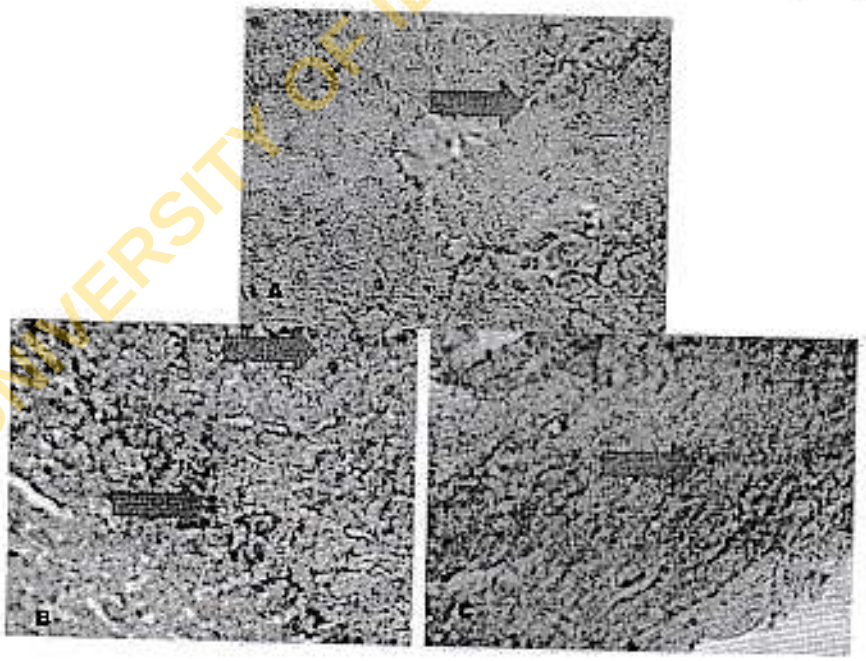

Fig. 15. Immunohistochemistry of c- reacting protein in heart of isoproterenol induced myocardial infarction rats. A (Control): show positive and low expression of CRP, B (ISO): shows higher expression of CRP than control, C (enalapril) shows lower expression of CRP than B (ISO). The slides were counterstained with high definition haematoxylin. Mag. x100

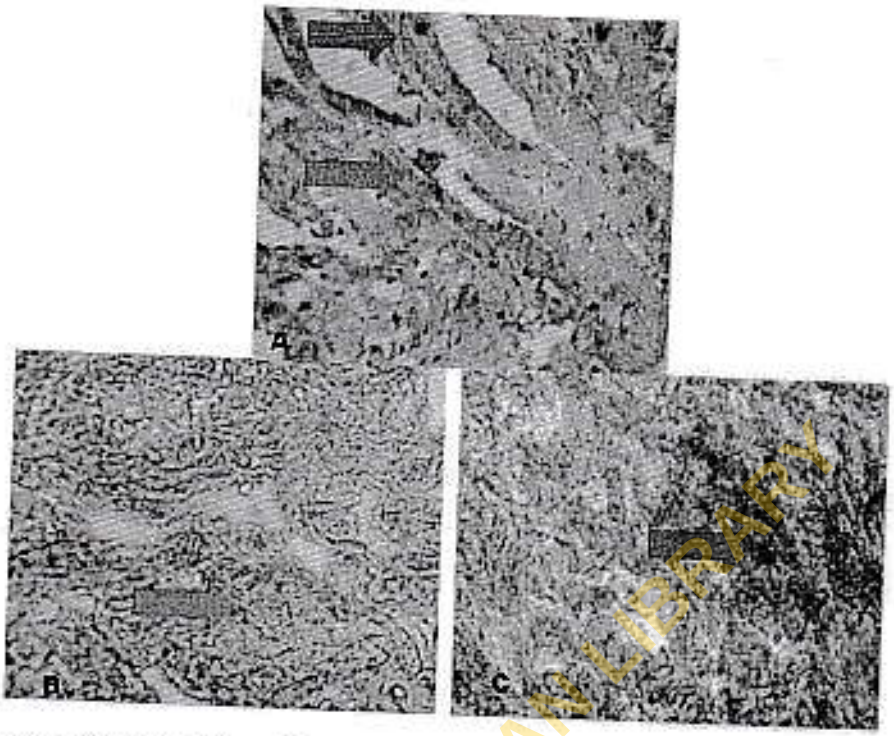

Fig. 16. Immunohistochemistry of interleukin-10 in heart of isoproterenol induced myocardial infarction rats. A (Control): show positive and higher expression of IL-10, B (ISO): shows lower expression of IL-10 than control, C (enalapril) shows higher expression of IL-10 than B (ISO). The slides were counterstained with high definition haematoxylin. Mag. x100

Angiotensin converting enzyme inhibitors are known to prevent both the generation of the potent vasoconstrictor angiotensin II and degradation of the powerful vasodilator bradykinin, which promotes endothelial cell release of NO [30]. In this study, rats treated with ISO had significant decreases in blood pressure parameters (SBD, DBP and MAP) when compared with the controls. This was however prevented in the ENA-treated group. There have been earlier reports of hypotension in subjects with acute myocardial infarction [31,32]. From this study, it was interesting to observe that ENA, a known antihypertensive drug, was able to preserve the blood pressure measurements of ISO-treated rats comparable to the controls. This might have been a consequence of its ability to prevent myocardial infarction. Studies have actually shown that ACEIs have been used in the management of myocardial infarction [33,34,35]. Isoproterenol, a β-adrenergic agonist is known to produce stress in the myocardium due to the generation of free radicals by its auto-oxidation. Some of the mechanisms proposed to explain its damage to cardiac myocytes include coronary hypotension, calcium overload, hypoxia, energy depletion and excessive production of free radicals as a result of catecholamine autoxidation [36,37,38]. 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 [39], it was concluded that in patients suffering with ischaemic 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 ischaemic events that could be incurred as a result of low blood pressure. The enalapril used in this study was able to restore the haemodynamic changes caused by isoproterenol indicating its ability to protect against establishment of myocardial infarction.

In this study, the results of haematological analysis showed that ISO caused significant increase in the levels of WBC, PCV, MCV and MCH while ENA caused significant decrease in WBC and no changes in the erythrocyte indices relative to control. The increase in the level of WBC could be explained in terms of necrosis

caused by ISO leading to white blood cell mobilization [11]. The significant reduction in the level of this parameter by enalapril could also be seen as its ability to counteract the toxic effect of isoproterenol.

The toxicant also caused significant increase in the levels of AST and ALT while ENA caused significant decrease in the levels of these enzymes. In heart failure, the heart has an impaired ability to deliver blood to the body and may in the process affects the kidney and liver. The liver can become dysfunctional, and liver enzymes can be released into the blood [40]. It thus means that the increases noted for the liver enzymes in this study implied that isoproterenol could impair liver functions and this was counteracted by enalapril indicating that enalapril has beneficial effect beyond being an ACE inhibitor.

It was also observed that ISO caused significant decrease in the level of NO while ENA caused significant increase. Nitric oxide (NO) is known to play important functional roles in a variety of physiological systems. For instance within the vasculature, NO induces vasodilation, inhibits platelet aggregation, prevents neutrophil/platelet adhesion to endothelial cells, inhibits smooth muscle cell proliferation and migration, regulates programmed cell death (apoptosis) and maintains endothelial cell barrier function [41]. Nitric oxide (NO) is known to be deficient in chronic progressive renal disease (CRD) and in end-stage renal disease (ESRD) [42,43] and this could result from arginine deficiency [44] which may be caused by a loss of functional renal mass, increased endogenous NO synthase (NOS) inhibitors that accumulate in renal failure [44], and/or other causes, such as increased oxidant stress [45]. Low NO production may also contribute to and/or exacerbate the progression of CRD by both hemodynamic and renal growth-promoting actions [46]. It should also be noted that NO blockade can lead to increased blood pressure and attenuated or delayed the hypotensive effect of all ACE inhibitors [47]. ACE inhibitors such as enalapril also augment the hemodynamic vasodilator action of bradykinin [48]. The increased level of NO in this study due to enalapril may further affirm its antihypertensive property and hence cardioprotective effect.

ISO caused significant increase in the levels of oxidative stress markers such as MDA, H_2O_2 and MPO while ENA caused significant decrease in the levels of these markers in a similar fashion to the control. Oxidative stress constitutes an alteration produced by disequilibrium between generation of free radicals (FR) and the antioxidant system, which can lead to a damage state, in particular of the biomolecules [49,50,51, 52,53]. FR generates the lipid peroxidation process in an organism with malondialdehyde (MDA) level used as a marker of oxidative stress [54]. Myeloperoxidase (MPO) is abundant in the granules of inflammatory cells and it is an important enzyme in the generation of reactive oxygen species (ROS) [55,56,57]. Hydrogen peroxide (H_2O_2), an ROS marker has been suggested as a mediator of vascular structural and functional alterations observed in hypertension [58,59,60,61,62]. The reduction of these oxidative markers by enalapril is a pointer to its ability to scavenge the radicals generated by the toxicant and it thus showed that enalapril has anti-oxidant activity. In fact, De Cavanagh et al. [63] reported that enalapril inhibits free radical formation and attenuates oxidative stress and also prevents damage to the liver and kidney. This was further confirmed by the ability of this ACE inhibitor to increase the levels of antioxidant enzymes such as SOD, GPx, GST and GSH evaluated in this study. This view is clearly supported by a study carried out by Chandra et al. [64], where it was concluded that enalapril has anti-oxidative property and this may have been responsible for its cardioprotective property. As a matter of fact, ENA caused a significant increase in the levels of protein thiols and non-protein thiols further confirming its anti-oxidant property. The thiol compounds function in the maintenance of cellular redox balance and their play important role in controlling oxidative stress [65,66,67].

Cells have evolved several antioxidant strategies aimed at the detoxification of ROS with glutathione redox cycle as one of the major protective systems against oxidant damage. This cycle composed of the enzymes glutathione peroxidase (GPx) and glutathione reductase (GSSG-Rd) and the co-substrates glutathione and NADPH [68]. Glutathione is the most abundant non-protein intracellular thiol, and has a multiple role as an antioxidant agent [69]. Though the mechanism(s) underlying the enhancement of glutathione and glutathione-related enzymes by ACEI remains unknown, however, tissue glutathione levels and GSSG-Rd and GPx activities have been shown to increase in response to experimentally induced oxidative stress [70].

In this study, histopathological examinations showed that while there was severe infiltration of inflammatory cells into the cardiac tissue of the ISO group, there was no visible lesion seen in the ENA and control groups (Fig. 13). This increase in the inflammatory cells may have been responsible for the increase in the levels of WBC noted in this study (Table 1). It should be noted that the isoproterenol-induced myocardial alterations are similar in certain respects to those occurring in human beings following a myocardial infarction [71]. It is thought that the β-adrenergic cardiostimulatory activity exerted by ISO increases cardiac oxidative metabolism to a level that exceeds the amount of oxygen available to the myocytes through the unobstructed coronary circulation. The area of the heart most susceptible to hypoxia caused by tachycardia appears to be the left ventricular subendocardium [72,73]. Myocyte damage observed following exposure to ISO includes both apoptosis and necrosis [74]. In the study on the isoproterenol-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 [75]. In our study, enalapril did not show any visible cardiac tissue damage possibly through its ability to prevent cell infiltration thus preventing apoptosis and necrosis.

The immunohistochemical analysis showed that there were high expressions of cardiac troponin and CRP in ISO group but lower expression of these proteins in ENA and control groups (Figs. 14 and 15). In the case of IL-10B, there was low expression of this protein in ISO group but higher expression in ENA and control groups (Fig. 16). Cardiac troponins are regulatory proteins within the myocardium that are released into the circulation when damage to the myocyte has occurred. Therefore, serum troponin is an exquisitely sensitive marker of myocardial injury and is necessary for establishing the diagnosis of MI [76,77,78]. This study has shown that ISO caused myocardial injury with upregulation of this biomarker. On the other hand, the down regulation of cardiac troponin by ENA also showed that this drug has ability to protect against myocardial injury in rats.

C-reactive protein (CRP) has the capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumonia*. It was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage [79]. It is a known fact that tissue necrosis is a potent acute-phase stimulus. In myocardial infarction, there is a major CRP response with the magnitude of this response indicating the extent of myocardial necrosis [80]. In all acute myocardial infarcts, CRP is co-deposited with activated complement [81,82], and research findings have shown that the CRP response did not only reflects tissue damage in this context but also may actually contribute significantly to the severity of ischemic myocardial injury [83]. The lowering of the level of CRP in this study by ENA is a pointer to its ability to halt cardiovascular disease hence cardioprotective effect through its anti-oxidant and anti-inflammatory properties.

Immunohistochemistry in this study further showed that ENA caused increased level of IL-10B. IL-10B is a Th_2-type cytokine that is produced by a wide range of immunological cell types, including monocytes/macrophages, and it is a potent inhibitor of the proinflammatory cytokines and chemokines [84]. Studies have shown that endogenous IL-10 limits angiotensin II (ANG II)-mediated oxidative stress, inflammation and vascular dysfunction both *in vivo* and *in vitro*, indicating a protective action of IL-10 in vascular diseases such as arterial hypertension [85]. As a matter of fact, IL-10 attenuates the increases in vascular superoxide and endothelial dysfunction during diabetes and atherosclerosis [86,87]. In the same way, it could be suggested that IL-10 might be a mediator of cardiac protection against arterial hypertension. It thus shows that the cardioprotective effect of enalapril may also be linked to its anti-inflammatory property as shown by the up regulation of IL-10.

5. CONCLUSION

In conclusion, this study has shown that enalapril, an ACE inhibitor has cardioprotective properties, which it exhibited through its anti-oxidant, anti-inflammatory and anti-apoptotic effects. Its antihypertensive property is also exhibited through its nitric oxide increasing ability leading to vasodilation and hence decreases in peripheral resistance.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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