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Corchorus olitorius Extract Attenuate Isoproterenol-Induced Cardiac Injury via Inhibition of Oxidative Stress, Arrhythmia and Pro-Apoptotic Protein Bax Expression

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BAA and TOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AO and IOEB managed the analyses of the study. Authors OI, OF, AA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: *Corchorus olitorius* (CO) leaf was reported to possess abundant antioxidants and used in the traditional treatment of heart disease. Previous studies revealed the protective roles of antioxidants against oxidative stress and inflammation, which are important underlying pathogenesis of myocardial injury that leads to infarction and heart failure. Therefore, CO leaf was chosen to evaluate its cardio-protective effects against ischemic-induced myocardial injury.

Materials and Methods: Thirty male rats (Wistar strains) were divided into five groups (n = 6): normal control group, myocardial injury control group, pretreatment groups (250 and 500 mg/kg), positive control group (10 mg/kg enalapril). After pre-treatment of rats with ethanol leaf extract of *C*O for 19 days, Isoprenaline (100 mg/kg) administration induced acute myocardial injury and parameters like blood pressure, electrocardiogram, lipid peroxidation, antioxidants were assessed and tissue subjected to histological evaluations.

Results: Isoproterenol given through subcutaneous significantly ($p<0.05$) reduced blood pressure and electrocardiography showed reduced p-interval and prolongation of QRS-interval in rats. The extract significantly increased the blood pressure and p-interval, QRS-interval were significantly reduced. The significant increase in tissue malondialdehyde, serum myeloperoxidase, creatine kinase-MB, lactate dehydrogenase and expression of Bax in the infarction control rats was decreased ($p<0.05$) in pre-treatment rats. Pre-treatment also increased glutathione-s-transferase, reduced glutathione and non-protein thiol level significantly. In contrast to cardio-injury control, histology showed mild level of inflammation and fatty infiltration in pre-treated rats.

Conclusion: This study showed the protective role of ethanol extract of CO against myocardial injury through anti-apoptotic, antioxidant, anti-inflammatory and anti-arrhythmic effect.

Keywords: Corchorus olitorius; oxidative stress; inflammation; arrhythmia; apoptosis; immunohistochemistry.

1. INTRODUCTION

Cardiovascular disease has been described to be the deadliest disease, even more than Human Immunodeficiency Virus (HIV) and all forms of cancer except lung cancer and responsible for millions of death all over the world. Important cardiovascular related disease include hypertension (the most common), stroke, coronary artery disease like arteriosclerosis, arrhythmia, heart block, myocardial infarction (also called heart attack) and ventricular hypertrophy. Apart from stroke, almost all these disease conditions usually lead to heart failure with myocardial infarction referred to as the leading cause of heart failure and its associated death worldwide [1]. Acute myocardial infarction (AMI) is a major cause of heart attack that occur as a result of diminished coronary blood flow which results from coronary obstruction (arteriosclerosis). In 2008, this ischemic heart disease was responsible for about 7.3 million deaths all over the world according to World Health Organization. Review evidence has shown that the prevalence of acute myocardial infarction is greater in wealthier countries than developing countries [2] but gradually becoming prevalent in developing countries, probably due to acquisition of lifestyle related risk factors [3] and was reported to be responsible for about 150,000 death in developing country like Nigeria in 2017. Between 1990 and 2020, coronary heart disease has been anticipated to increase by 120% in women and 137% for men in developing countries [4]. Before myocardial infarction occur, coronary obstruction, hypoxia or ischemic insult to myocardial tissue results into generation of free radicals, release of inflammatory mediators, lipid peroxidation [5] which eventually trigger myocardial tissue death either through apoptosis or necrosis with apoptosis occurring within 6 – 8 hours after severe ischemia and secondary necrosis occurring between 12 hours to 4 days [6]. Activation of reactive oxygen species was explained to increase myocardial oxidative stress which is associated with lipid peroxidation characterized with enhanced tissue level of malondialdehyde, disruption of cell membrane integrity, activation of phospholipase AA which trigger strong inflammatory mediator (myeloperoxidase) release, infiltration of tissue with leucocytes amidst many other cascade of pathological reactions that is triggered [7]. Current drugs used in the management and

treatment of acute MI include thrombolytic drugs, anti-platelet drugs, antithrombin, beta blockers, statins, organic nitrates and angiotensin converting enzyme inhibitor [8]. These drugs improve reperfusion and enhance myocardial re-oxygenation but this effect is often associated with myocardial reperfusion injury and eventually result in death of patients involved. In addition, severe bleeding is a major toxic effect of thrombolytic, antiplatelet and antithrombin agents, which could sometimes be life threatening. During ischemia-induced myocardial injury, the release of reactive oxygen species and inflammatory mediators is known to trigger programmed cell death (apoptosis) and tissue necrosis and eventually constitute area of infarction. These important pathophysiology of myocardial injury can become targets for new drugs and reduce the risk associated with the use of current available drugs in the treatment of heart attack. This lead to substantial research focus on plants with phytochemicals (especially phenols and polyphenols) as a potential therapeutic target of this ischemic heart disease. One such plant is *Corchorus olitorius (L.),* a shrub from the Malvaceafamily, the leaves of which are rich in antioxidants, such as vitamin C, vitamin E, β-carotene, α-tocopherol, glutathione and phenols [9] and commonly called jute mallow in English, "ewedu" in the South Western Nigeria. *Corchorus olitorius* belongs to the kingdom: plantae, subkingdom: Viridiplantae, Infrakingdom: Streptophyta, Superdivision: Embyophyta, Division: Tracheophyta, Subdivision: Spermatophytina, Class: Magnoliopsida, Superorder: Rosanae, Order: Malvales, Family: Malvaceae and to the Genus Corchorus. The leaf extract of the plant is employed in folklore medicine in the treatment of gonorrhea, pain, fever and tumor [10]. Ethanol extracts of this plantwas shown to produce a significant hepato-protective effect by decreasing serum and liver levels of ALT, AST, and total protein at doses of 250 and 500 $mgkg^{-1}$ in carbon tetrachloride induced hepatotoxic rats [11]. Traditionally, the leaf twigs of this plant was reported to be used against heart trouble [12,13] and the protective role of *Corchorusolitorius* leaf extract against arsenic-induced myocardial injury was reported by Das and colleague [14]. Based on evidence from these studies, the present research was designed to evaluate the protective role of *Corchorusolitorius*leaf extract against oxidative stress, inflammation and apoptosis associated with Isoprenaline-induced myocardial injury the preceding phase before myocardial infarction in Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals used in this study include: thiobarbituric acid, hydrogen peroxide, reduced glutathione, Sodium azide, Tris-KCL, Trichloroacetic acid, DTNB 5', 5'-Dithiobis- (2-nitrobenzoate) , K_2HPO_4(Phosphate buffer), 0.1MPO_4 (Myeloperoxidase), Sulphosallicyclic acid, HCL (Hydrochloric acid), H_2SO_4 (Sulphuric acid), biurete's reagent, O-dianisidine,bicarbornate buffer obtained from sigma, Randox laboratory provided LDH Elisa Kit, Bcl-2-associated X protein antibody was purchased from Bioss Inc. Woburn, Massachusetts, USA, 10% formaldehyde solution for myocardial preservation. All other chemicals were of analytical grade that was obtained from British drug houses (Poole, UK).

2.2 Plant Specimen and Extract Preparation

Fresh healthy leaves of *Corchorus olitorius* CO) (local name 'ewedu' and English name Jute mallow) were collected, identified and authenticated at the Department of Forest Herbarium, Forestry Research Institute of Nigeria, Jericho Ibadan, Oyo State Nigeria. The voucher specimen FHI number given at the Forest Herbarium is 112603. The leaves were air dried for 21 days and pulverized into powdery form with high capacity grinder and soaked into 70% aqueous ethanol at the ratio of 1:10 (w/v) for 3 days. The extract was filtered using Whitman filter paper and evaporated to dryness under reduced pressure until only water layer remains. Remaining water was removed by rotary evaporator and semi-solid form was obtained.

2.3 Experimental Animals

Male Wistar rats, weighing 120-150 g, were used in the study. The rats were fed with commercial rat chow and water liberally. The light/dark cycle of 12 hours was ensured and acclimatization was done for one week before administration of extract and isoproterenol.

2.4 Experimental Design

Animals were randomly divided into five groups comprising of six animals in each group. Group I (normal control) received distilled water orally for

21days, group II (myocardial injury/ infarction) received distilled water orally for 19 days and Isoprenaline administered on day 20 and 21 subcutaneously. Group III, IV and V were given 250, 500 mg/kg extract and 10 mg/kg enalapril respectively for 19 days before Isoprenaline-induced myocardial injury on day 20 and 21 (100 mg/kg S.C).

2.5 Blood Pressure Measurement

The systolic pressure, diastolic pressure, mean arterial pressure, tail blood flow, tail blood volume and heart rate were measured using Kent Incorporation non-invasive blood pressure monitoring machine without anesthesia.

2.6 Electrocardiography

The electrocardiographic evaluation of the rats was done using a 6 lead computer ECG machine, EDAN 1010. The ECG machine was set at 50 mm/s paper speed and 10 mm/mv voltage calibrations. The procedure was carried out, as described by Calderon et al. [15].

2.7 Biochemical Estimation

Animals were sacrificed through cervical dislocation after the collection of blood samples from the retro-orbital plexus and the heart was excised and rinsed in cold saline. The heart tissue was dissected longitudinally, weighed and a section was fixed in 10% formalin for histopathological examination. The other longitudinal section was immediately homogenized in cold 0.1M Tris-HCl buffer (pH 7.4, 1:10 w/v). The homogenate was then centrifuged (Cold centrifuge) at 13,000 rpm for 10min at 4^{o}C and the supernatant was stored at -80^{o}C for further biochemical analysis. Myocardial tissue malondialdehyde (MDA) level, an index of lipid peroxidation was determined by measuring the formation of the thiobarbituric acid reactive substances (TBARS) according to the method described by Olszewska-Słonina et al., [16], Reduced glutathione (GSH) was determined at 412 nm using the method described by Beutler et al., [17], Glutathione peroxidase (GPx) activity was measured according to Wendel et al., [18] and Glutathione-S-transferase (GST) was estimated by following the method of Habig et al., [19]. Protein was estimated based on Lowry et al., [20] protocol. Serum level of lactate dehydrogenase and creatine kinase-MB was based on Randox kit protocol and serum myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation, was determined according to the modified method of Xia and Zweier [21].

2.8 Immuno-Histochemistry of Bcl-2-Associated X Protein

Immunohistochemistry of paraffin embedded myocardial tissues were carried out after the tissues were preserved with formalin as described earlier by Alabi et al., [22].

2.9 Histology of Myocardial Tissue

The heart tissues obtained from all experimental group were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissues were processed, dehydrated and embedding in paraffin. Then the tissues were stained with hematoxylin and eosin [23] and examined under high power microscope and photomicrographs were taken.

2.10 Statistical Analysis

All the values were reported as the mean $\pm$ standard error of mean. The difference between two groups were compared using paired t-test and analysis of variance was used for comparism within the group with Tukey's post-hoc test. Statistical analysis were carried out using GraphPad Prism 5.0 and the level of significance was taken as $p<0.05$.

3. RESULTS

3.1 Hemodynamics

Isoproterenol administration caused a significant reduction in systolic, diastolic and mean arterial pressure ($p<0.05$) in the myocardial infarction rats compared with non-infarction control. The rats pretreated with extracts and enalapril maintained the normal systolic, diastolic and mean arterial pressure (Fig. 1). Associated with severe hypotension within the infarction study rats was a significant low tail blood volume and tail blood flow. Blood volume and blood flow was maintained in the Pretreatment groups (Fig. 2).

3.2 Electrocardiogram

From Table 1, pretreating the rats protect their heart against prolonged QRS interval, shortened P interval and low R amplitude associated with the isoproterenol induced acute myocardial infarction.

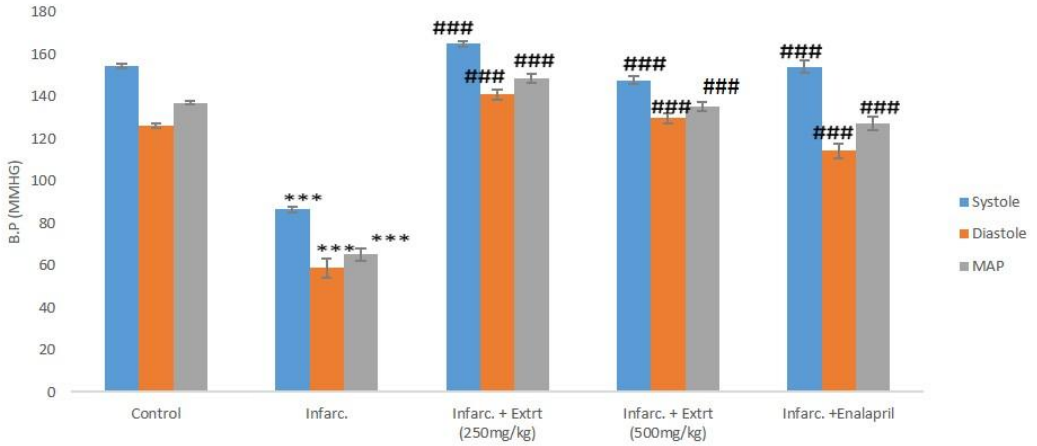

Fig. 1. The effect of myocardial injury,extract and enalapril on the systolic, diastolic and mean pressure of experimental rats expressed in bar chart

*Values were expressed as mean ±SEM, for six rats in each group.*Values differ significantly from normal control (*p<0.05, **p<0.01, ***p<0.001). #Values differ significantly from infarction (#p<0.05, ##p<0.01, ###p<0.001)*

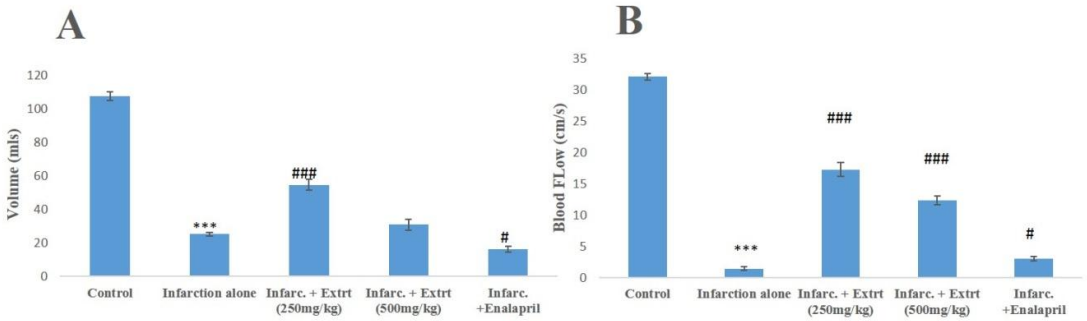

Fig. 2. The effect of myocardial injury, extract and enalapril on tht tail blood volume (A) and blood flow (B) of experimental rats expressed in the bar chart

*Values were expressed as mean ±SEM, for six rats in each group.*Values differ significantly from normal control (*p<0.05, **p<0.01, ***p<0.001). #Values differ significantly from isoproterenol control (#p<0.05, ##p<0.01, ###p<0.001)*

Table 1. The effect of myocardial injury/infarction, extract and enalapril on ECG parameters (QRS Interval, P interval & R amplitude) and myocardial pro-apoptotic protein (Bax) expression of experimental rats

Treatment group	QRS Interval (m/s)	P Interval (m/s)	R amplitude (mv)	Bax expression area (%)
Control	13 ± 0.63	21.8 ± 0.66	0.4324 ± 0.01	5.70
Infarction	21.4 ± 0.93**	12.6 ± 0.51***	0.1892 ± 0.02	77.50**
Infarction + Extract (250 mg/kg)	12 ± 0.55##	19.2 ± 1.60#	0.374 ± 0.04##	45.60#
Infarction + Extract (500 mg/kg)	15 ± 0.32##	19.6 ± 0.68##	0.1778 ± 0.01	46.22#
Enalapril	15.4 ± 0.40##	22.8 ± 0.58###	0.1874 ± 0.01	56.45#

*Values were expressed as mean ± SEM, for six rats in each group. *Values differ significantly from normal control (*p<0.05, **p<0.01, *** p<0.001). # Values differ significantly from isoproterenol control (#p<0.05, ##p<0.01, ### p<0.001)*

3.3 Biochemical Study

The level of serum LDH and CK-MB were increased significantly in the infarction group and low in the pretreatment groups (Fig. 3).

The serum level of MPO and tissue MDA that was increased during myocardial infarction was significantly reduced when rats were pretreated before inducing infarction (Fig. 4). Glutathione (GSH) and non-protein thiol level (NPT) level in the myocardial tissue was reduced during infarction and significantly increased when rats where pretreated with extract and enalapril (Fig. 5). There was no significant effect of acute myocardial infarction and pretreatment on enzymes like glutathione peroxidase and glutathione-s-transferase (Fig. 4).

3.4 Immunohistochemistry

There was a significant increase in tissue expression of bax in the cardiac injury control group. Pretreatment of rats with 250 mg/kg, 500 mg/kg and enalapril decreased the expression of this protein significantly (Fig. 6 & Table 1).

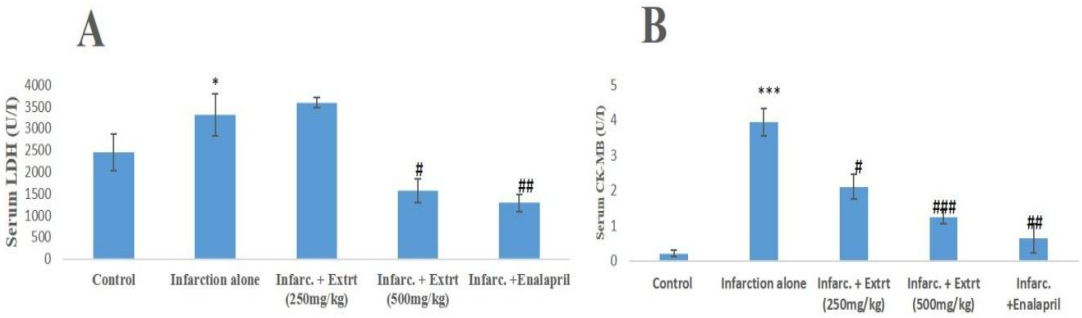

Fig. 3.The effect of myocardial injury, extract and enalapril on the Seram Lactate dehydrogenase (A) and Creatine Kinase-MB (B) level of experimental rats expressed in bar chart

*Values were expressed as mean ±SEM, for six rats in each group.*Values differ significantly from normal control (*p<0.05, **p<0.01, ***p<0.001). #Values differ significantly from infarction (#p<0.05, ##p<0.01, ###p<0.001)*

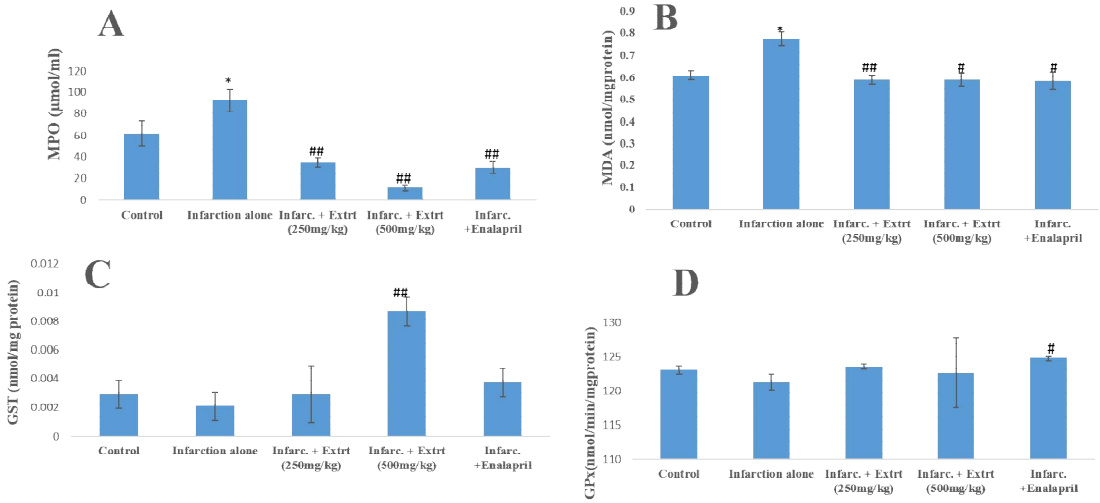

Fig. 4. The effect of myocardial injury, extract and enalapril on the Tissue MPO (A), MDA (B), GST (C), GPx (D) level of experimental rats expressed in bar chart

*Values were expressed as mean ±SEM, for six rats in each group.*Values differ significantly from normal control (*p<0.05, **p<0.01, ***p<0.001). #Values differ significantly from infarction (#p<0.05, ##p<0.01, ###p<0.001)*

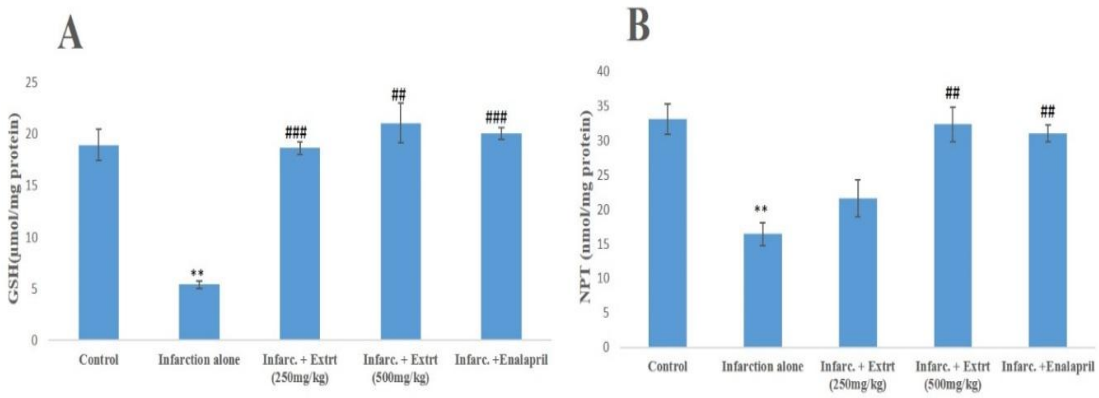

Fig. 5.The effect of myocardial injury, extract and enalapril on the Tissue GSH (A), and NTP (B) level of experimental rats expressed in bar chart

*Values were expressed as mean ±SEM, for six rats in each group.*Values differ significantly from normal control (*p<0.05, **p<0.01, ***p<0.001). #Values differ significantly from infarction (#p<0.05, ##p<0.01, ###p<0.001)*

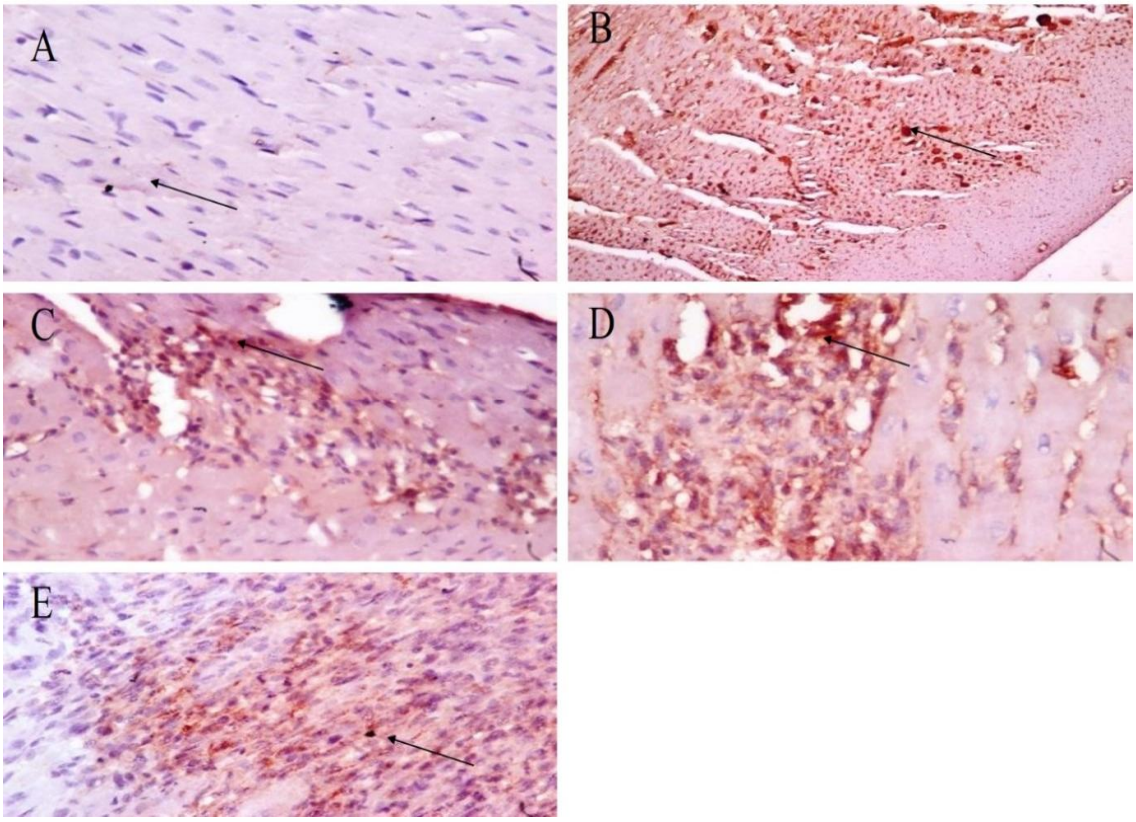

Fig. 6. Photomicrograph showing bax expression (mag. x400): A (control) show mild expression of bax. B (Myocardial injury alone) severe expression of bax.C & D (250 and 500 mg/kg extract pretreatment) very mild expression. E (10 mg/kg enalapril) very mild expression

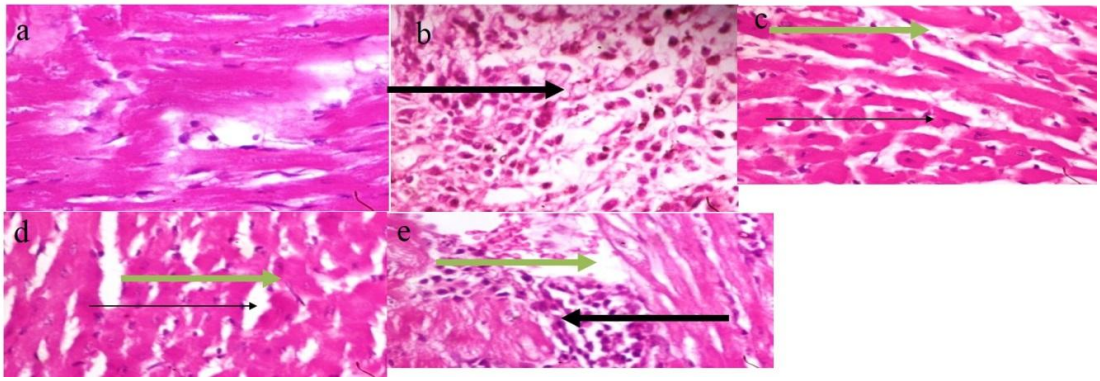

Fig. 7. Histology of the myocardium x400: a (control) no significant myocardial lesion. b (injury/infarction alone) severe extensive multifocal area of myocardial infaraction with marked infiltration of the myocardium and pericardium by inflammatory cells (black arrow). C & D (250 and 500 mg/kg extract pretreatment) moderate disseminated infiltration of the myocardium (slender arrows) and mild fatty infiltration (green arrow). e (10 mg/kg enalapril) moderate focal area of myocardial infarction with inflammatory cell and fatty infiltration

3.5 Myocardial Tissue Histology

Myocardial infarction tissue section revealed the multifocal infiltration of myocardium with white blood cells and was very mild in all the pretreatment groups (Fig. 7).

4. DISCUSSION

The obstruction of blood supply to cardiac muscle through coronary artery (either partial or complete obstruction) is known to trigger myocardial injury and eventually results in myocardial infarction. The ischemic tissue injury activate the production of reactive oxygen species and activation of pro-inflammatory mediators that can cause death of myocardial tissue apoptosis and necrosis [4,6]. From the present study, subcutaneous administration of 100 mg/kg isoproterenol for two days induced myocardial tissue injury, through the significant elevation of myocardial MDA, serum MPO and significant reduction in the tissue level of myocardial GSH and NPT. The pre-treatment of rats with 250 mg/kg and 500 mg/kg CO extract alleviate myocardial injury by significantly increasing the tissue level of GSH and NPT. This is associated with significantly reduced tissue level of MDA and serum level of MPO. The GSH is a known intracellular non-enzyme antioxidant and a thiol-containing compound that helps to detoxify hydrogen peroxide (H_2O_2)in the presence of glutathione peroxidase (antioxidant enzyme) [24] during ischemia-induced oxidative stress, thereby preventing the production and release of free radicals through membranes. These suggest that CO leaf extract possess antioxidant activity, which can protect the cardiac tissue against Ischemia-induced oxidative stress during myocardial injury. To support the antioxidant effect of the extract through enhanced GSH level, tissue non-protein thiol (NPT) level was also elevated significantly. Although the GSH is the major NPT in the cell [25], other forms of NPT like N-acetyl-cysteine (NCSH) and taurine are important intracellular antioxidants [26,27,28]. Similarly, pre-treatment of rats with CO extract reduced the tissue level of MDA a marker of oxidative stress associated with lipid peroxidation [29]. The results obtained from the present study on GSH, NPT, GPx and MDA is similar to the report of Das et al., [14], who studied the antioxidant effect of CO leaf extract against arsenic-induced myocardial injury in rats.

The tissue level of MDA, serum LDH and CK-MB have been revealed to be severely increased during Isoproterenol-induced myocardial injury by Sunanda, [30] and Roza et al., [31]. From the present study, increased cardiac tissue level of MDA in the infarction control rats reflects lipid peroxidation that lead to alteration of myocardial membrane integrity and release of markers of myocardial injury like LDH and CK-MB in excess amount [32] into the serum. Possibly through the inhibition of lipid peroxidation, pre-treatment of rats with CO leaf extract significantly reduced the serum level of LDH and CK-MB.

Serum level of MPO was significantly high in the infarction control rats and the pretreatment of

rats with CO leaf extract and enalapril reduced the level of this pro-oxidant and inflammatory marker. MPO is a member of heme peroxidase superfamily of enzymes and it generates a large number of reactive oxygen species that can also trigger lipid peroxidation and promote the post-translational modifications of target proteins [33]. Secreted by neutrophils, monocytes and certain tissue macrophages, MPO, as part of its normal host defense mechanism, generates a lot of reactive oxygen and nitrogen species that are essential in pathogen destruction [34]. Myocardial infiltration of MPO strongly indicate inflammation and oxidative stress and the low serum level of this marker in the pretreated rats revealed the anti-inflammatory effect of CO leaf extract and further confirm protective role of the extract against myocardial injury through the anti-oxidant effect.

The blood pressure and electrocardiogram were taken, few hours after 2 day injection of 100 mg/kg Isoproterenol. The results obtained revealed a significant decrease in the systolic, diastolic and mean arterial blood pressure in the infarction control rats compared with normal control rats ($p<0.05$). The reduced systolic, diastolic and mean arterial blood pressure observed in the infarction control rats showed the hypotensive effect of Isoproterenol previously described by Chagoya de Sanchez et al., [35]. From the result obtained in this study, severe hypotension associated with Isoproterenol-induced myocardial injury can be linked with cardiac output and peripheral resistance alteration. The cardiac output was altered, probably due to increased myocardial oxygen consumption arising from larger dose of Isoproterenol which possess higher affinity for β_1 and β_2 receptor of the myocardial tissue [36] leading to imbalance between cardiac muscle workload and oxygen supply [37]. This is associated with tachycardia, thereby reducing the ventricular filling time of the diastolic phase during cardiac cycle, leading to low stroke volume/ejection fraction and reduced cardiac output. In addition, vasodilator effect of Isoproterenol can reduce the peripheral resistance significantly and this can be responsible for the significantly reduced blood volume in the infarction control group.Pre-treatment of rats with 250 and 500 mg/kg CO leaf extract and 10 mg/kg enalapril increased the blood pressure across systole, diastole and mean arterial pressure and reversed the hypotension. This result suggest that CO leaf extract protected the myocardium against the severe hypotension associated with Isoproterenol-induced myocardial injury in rats.

The electrocardiogram revealed a significantly prolonged QRS-interval and short p-wave interval in the infarction control rats. Wide QRS complex is a major reflection of intra-ventricular conduction disturbance that can be observed in right and left bundle branch blocks, heart failure and myocardial ischemia which can also reduce the cardiac output. Wide QRS complexes can also be seen after treating rats with several drugs like doxorubicin [38], disopyramide [39] and azithromycin [40]. From the result obtained, pretreating the rats with extract and enalapril protected the cardiac tissue against ischemia-induced arrhythmia by decreasing QRS-interval (ventricular depolarization), increasing; interval of P-wave (atrial depolarization) and R-amp (intensity of ventricular depolarization). The Bax is a pro-apoptotic protein of the Bcl-2 family that act on the membrane of cell mitochondria to promote the release of cytochrome C, to activate apoptotic initiating caspases and other execution caspases necessary for apoptotic signals [41]. The pretreatment of rats with 250 and 500 mg/kg ethanol extract decrease the expression of this pro-apoptotic protein significantly and this suggests the anti-apoptotic effect of the extract against isoprenaline-induced myocardial injury. The severe expression associated with the injury/infarction control group further support this.

5. CONCLUSION

This study revealed the protective effect of *Corchorus olitorius* ethanol leaf extract against Isoproterenol-induced myocardial injury through the antioxidant, anti-inflammatory, anti-hypotensive and anti-arrhythmic effect. Therefore, further studies on the phytochemicals of this plant may be beneficial in the treatment/management of acute myocardial infarction.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As for ethical approval, the research in relation to animal use has complied with all the necessary national regulations and institutional policies for the care and use of animals and ethical approval number given was UI-ACUREC/18/0057.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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