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Research Article
Earthworm Extract Exhibits Therapeutic Effect Against Epinephrine-Induced Myocardial Infarction in Rats

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Abstract

Background and Objective: Earthworms are oligochaete soil macroinvertebrates that play an essential role in soil fertility and production throughout time. In the present study, an attempt has been made to assess the therapeutic effects of earthworm extract against myocardial infarction induced by epinephrine injection in rats. **Materials and Methods:** Myocardial infarction was induced in rats by injecting 2 mg kg^{-1} epinephrine subcutaneously daily for 2 days. The rats were split into three groups: Control, MI and MI were given earthworm extract (60 mg kg^{-1}, orally) for 7 days. **Results:** The treatment with earthworm extract restored ST-segment near normal, improved cardiac troponin T, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total proteins, albumin, creatinine, uric acid, urea, malondialdehyde, nitric oxide, reduced glutathione, catalase, glutathione-S-transferase. According to the histological study, the earthworm extract-treated group exhibited remarkable improvement in the studied heart muscles, liver and kidneys in various areas. **Conclusion:** Earthworm extract possessed a therapeutic effect against epinephrine-induced myocardial infarction. Earthworm extract improves cardiac, liver and renal biomarkers and restores the EEG pattern to its original state. The therapeutic effect of earthworm extract results from its antioxidant and anti-inflammatory activities.

Key words: Epinephrine, myocardial infarction, ischemic heart disease, antioxidant, earthworm, oxidative stress, histopathology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular disease (CVD) is the primary cause of mortality worldwide[1]. The CVD encompasses a wide range of ailments, including heart and blood vessel abnormalities, Angina, MI and heart failure[2,3].

Myocardial infarction (MI) is an abrupt and prolonged decrease in myocardial blood flow that results in myocardial necrosis. It has marked by chest aches or discomfort that might spread to the shoulder, arm, back, neck or jaw[4,5]. For many years, MI has been one of the most frequent disorders seen in clinical practice and its prevalence has risen dramatically in recent years[6].

The pathogenic mechanism of myocardial injury is still not completely understood, however, the significance of oxygen-derived free radicals in myocardial injury has been demonstrated[7]. Overproduction of reactive oxygen species (ROS) leads to cell membrane damage in the ischemic myocardium, resulting in contractile failure and finally acute MI[8,9]. As a result, treatment therapies with antioxidant or free radical scavenging action may help to reduce oxidative stress linked to IHD.

Epinephrine, a catecholamine secreted by the adrenal medulla, is one of the neurohormones implicated with cardiac stress, responsible for the "fight or flight" response[10]. It is also utilized in the emergency room to treat acute respiratory distress in patients with croup syndrome or bronchiolitis[11,12]. On the other hand, in high doses, Epinephrine was found to play a vital role in generating the highly cytotoxic reactive oxygen species (ROS) and depletion of the antioxidant enzyme associated with MI[8]. The ROS act directly on cells, causing lipid, protein and DNA damage with overproduction of NO_2 and nitrosative derivatives and eventually cardiomyocytes damage[12].

Many current medications, such as organic nitrates, calcium channel antagonists and blockers, help avoid cardiovascular illnesses, but their usage is generally restricted due to side effects and negative responses[13]. This is one of the reasons why many people all around the world, especially those in wealthy nations are turning to complementary and alternative medicine, which includes natural products. Plants and animals can provide natural products for use as natural medicines. The earthworm is one of the natural medications available[14]. The earthworm is an oligochaete soil macro invertebrate that serves a critical role in soils' long-term fertility and productivity[15]. It has been widely used in traditional medicine around the world, especially in Asia, including India, Myanmar, China, Korea and Vietnam to treat fever, stomach discomfort, neck pain, brain diseases and digestive problems due to its multiple therapeutic characteristics[16,17]. Earthworm extract displays unusual antioxidant properties due to containing polyphenols[18].

In the present study, an attempt has been made to assess the therapeutic effects of earthworm extract against myocardial infarction induced by epinephrine injection in rats.

MATERIALS AND METHODS

Study area: The experiments were performed at Cairo University between January and March, 2022.

Chemicals and reagents: The MISR Company provided Epinephrine for pharmaceuticals. All the other chemicals utilized were obtained from the Biodiagnostic Company (El Motor St, Dokki, EGY).

Collection of earthworms: Earthworms were gathered from commercial vermiculture in the Giza Governorate and kept in plastic tubs with decomposed organic debris until they were employed for research.

Earthworm extract preparation: According to Dewi *et al.*[19], the whole extracted collected earthworm (1 kg) was rinsed with running water to remove the mucous from the surface Earthworms were immersed in distilled water for 6 hrs to remove dirt from their bodies with a continuous water exchange. After being cleaned, worms are then mashed into small pieces, smoothed and moved into a glass tube. About 80% of ethanol was added for 2 days and then evaporated in a water bath. The extract was then centrifuged at 3000×g for 10 min. The supernatant is filtered and then lyophilized (Christ freeze dryer) to get the extract.

Experimental animals: Adult male Wistar rats (Rattus norvegicus), weighing 190-200 g, were selected for the experiments. Ten rats were used to determine LD_{50}, while eighteen rats were used for the main experiment. Rats were purchased from the National Research Center (NRC, Dokki, Giza). Animals were kept in hygienic polycarbonate boxes (6 rats per box), in the well-ventilated animal house of the Department of Zoology, Faculty of Science, Cairo University, Egypt. They were supplied with a standard laboratory diet and water *ad libitum*. The animals were housed under suitable conditions and handled a 12/12 hrs light-dark cycle at (22-25) room temperature. Before the experiment began, the animals were housed in the laboratory for 7 days to acclimate to laboratory conditions.

Ethical consideration: The Cairo University, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt) (CU/I/F/64/20) approved the study's experimental protocols and procedures. All the experiments were carried out under international guidelines for the care and use of laboratory animals.

Acute toxicity study (LD_{50}): The LD_{50} of AcCF was determined according to the method described by Chinedu *et al.*[20].

Myocardial infarction induction: Myocardial infarction was induced in rats by injecting 2 mg kg^{-1} epinephrine subcutaneously once daily for 2 successive days[21].

Experimental design: Eighteen rats were divided into three groups (6/group) as the following:

- **Group 1 (control):** Rats were injected with physiological saline on the 1st and 2nd day at an interval of 24 hrs and then received distilled water orally for 7 days
- **Group 2 (MI):** Rats were injected with epinephrine (2 mg kg^{-1}, S.C.) on the 1st and 2nd day at an interval of 24 hrs then received distilled water orally for 7 days
- **Group 3 (E. worm extract):** Rats were injected with Epinephrine (2 mg kg^{-1}, S.C.) on the 1st and 2nd day at an interval of 24 hrs, then received earthworm extract (45 mg kg^{-1}, orally) for 7 days

Measurement of ECG: One day after the final dose of Epinephrine the rats were kept overnight fasting and then they were anaesthetized with light anaesthetic 3% sodium pentobarbital. Rats were placed supine and leads of the conventional electrocardiograph (ECG 100 G single-channel Electrocardiograph-china) were connected to the dermal layer of both front paws and hind legs for the measurement of ECG.

Animal handling and collection of the samples: The blood samples of the animals were immediately collected in sterile centrifuge tubes by the exsanguination method. The heart, kidney and liver were enucleated and transferred to a filter paper for removing blood traces. Pieces of the tissues were stored at -80 for biochemical analyses. In 10% formal saline, another piece of them was suspended for preparative fixation for Histopathological fixation.

Biochemical estimation: The accessible kits were used for the estimation of cardiac troponin T, creatine kinase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin, total protein, cholesterol, triglyceride (TG), creatine, uric acid and urea using biodiagnostic kits (Giza, Egypt).

Determination of oxidative stress parameters: In ice-cold 0.1 mol L^{-1} Tris-HCl buffers (pH 7.4), the heart, liver and kidney tissues were homogenized (10% w/v). The homogenate was centrifuged at 3000 g for 15 min at 40°C and the resultant supernatant was utilized to evaluate oxidative stress markers. Biodiagnostic kits were used to measure malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST) and nitric oxide (NO) according to the manufacturer's instructions (Giza, Egypt).

Histological examination: The heart, liver and kidney tissues were rapidly removed and embedded in paraffin, cut into 5 m sections and stained using hematoxylin and eosin (H&E). Photomicrographs were taken of the sections after they were examined under a light microscope.

Statistical analysis: Results were expressed as Means±SE. The comparisons within groups were carried out utilizing One-Way Analysis of Variance (ANOVA) with the Duncan *post hoc* test to compare the group means and $p<0.05$ was regarded statistically significant. The statistical analysis was performed using SPSS for Windows.

RESULTS

ECG patterns: The control rats showed a regular ECG pattern, whereas Epinephrine treated rats showed a marked ST-segment elevation. These changes were restored to near normal in earthworm extract treated rats (Fig. 1).

Cardiac function markers: There was a significant elevation ($p<0.05$) in the cardiac troponin T, creatine kinase, LDH and AST levels of the MI group compared to the control group. While significant decrease ($p<0.05$) was observed in the aforementioned parameters after treatment with earthworm extract compared with the MI group (Table 1).

Liver function markers: Compared with the control group, epinephrine-treated rats resulted in a significant increase ($p<0.05$) in levels of ALT, ALP and a decrease in TP. However, the treatment with earthworm extract ameliorates these changes in the ALT, ALP and TP (Table 2).

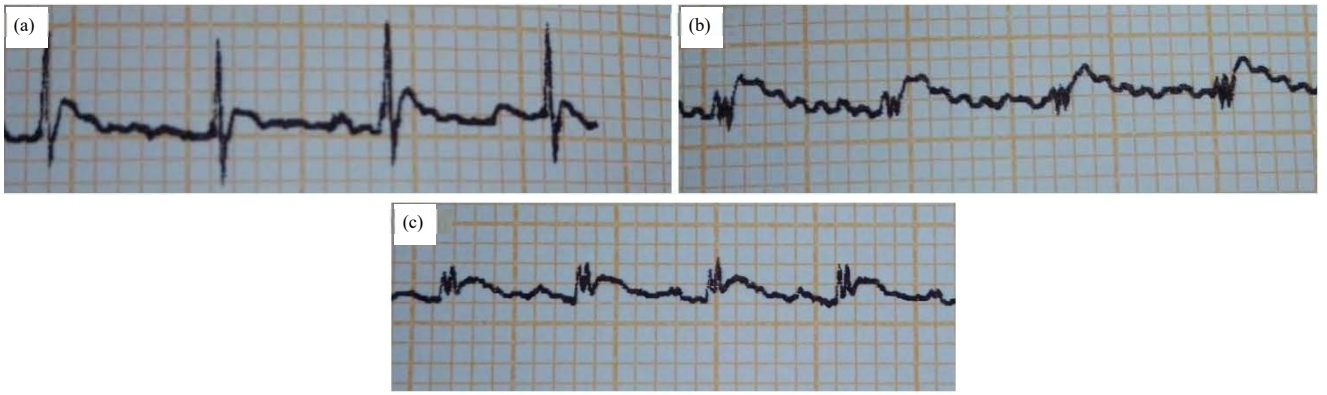

Fig. 1(a-c): ECG of the (a) Control group, (b) MI group and (c) Earthworm extract group

Table 1: Effect of E. worm on cardiac biomarkers of myocardial infarction rats

	Experimental groups		
Parameters	Control	MI	E. worm
cTn1 (ng mL^{-1})	0.12±0.00^{a}	1.11±0.03^{c}	0.51±0.05^{b}
LDH (U L^{-1})	1415.99±28.27^{a}	2703.39±92.92^{b}	1566.57±135.70^{a}
AST (U mL^{-1})	127.25±3.95^{a}	169.66±5.65^{b}	139.01±4.58^{a}
Creatine kinase-MB (ng mL^{-1})	665.97±29.80^{a}	1382.80±24.62^{b}	742.88± 33.65^{a}

Values are Mean±SE (n = 6 per group), each value not sharing a common letter superscript is significantly different (p<0.05)

Table 2: Effect of E. worm on liver functions of myocardial infarction rats

	Experimental groups		
Parameters	Control	MI	E. worm
ALT (U mL^{-1})	42.89±1.04^{a}	67.95±2.38^{b}	46.49±1.55^{a}
ALP (U L^{-1})	220.55±8.12^{a}	425.60±16.22^{c}	299.90±9.01^{b}
Albumin (g dL^{-1})	4.82±0.08^{b}	4.52±0.10^{c}	4.69±0.03^{a,b}
Total Protein (g dL^{-1})	6.91±0.12^{b}	6.26±0.16^{a}	6.45±0.25^{a,b}

Values are Mean±SE (n = 6 per group), each value not sharing a common letter superscript is significantly different (p<0.05)

Table 3: Effect of E. worm on lipid profile of myocardial infarction rats

	Experimental groups		
Parameters	Control	MI	E. worm
Cholesterol (mg dL^{-1})	72.85±3.49^{a}	77.28±3.39^{a}	74.49±3.38^{a}
TG (mg dL^{-1})	76.15±8.11^{a}	86.31±6.63^{a}	66.80±5.20^{a}

Values are Mean±SE (n = 6 per group), each value not sharing a common letter superscript is significantly different (p<0.05)

Table 4: Effect of E. worm on kidney functions of myocardial infarction rats

	Experimental groups		
Parameters	Control	MI	E. worm
Creatinine (mg dL^{-1})	0.75±0. 01^{a}	1.14±0.03^{c}	0.83±0.02^{b}
Urea (g dL^{-1})	55.74±1.99^{a}	76.40±1.32^{b}	60.55±2.55^{a}
Uric acid (mg dL^{-1})	1.55±0.04^{a}	2.18±0.14^{b}	1.59±0.05^{a}

Values are Mean±SE (n = 6 per group), each value not sharing a common letter superscript is significantly different (p<0.05)

Lipid profile: Table 3 showed a non-significant change (p>0.05) in cholesterol and TG concentration of the MI group compared to the control group. Similarly, cholesterol and TG concentration showed non-significant change (p>0.05) after earthworm extract administration compared with the MI group.

Kidney function markers: A significant increase (p<0.05) in the concentration of creatinine, uric acid and urea of the MI group compared to the control group was shown in Table 4. On the other hand, a significant decrease (p<0.05) was noticed in these parameters after the earthworm extract treatment compared with the MI group.

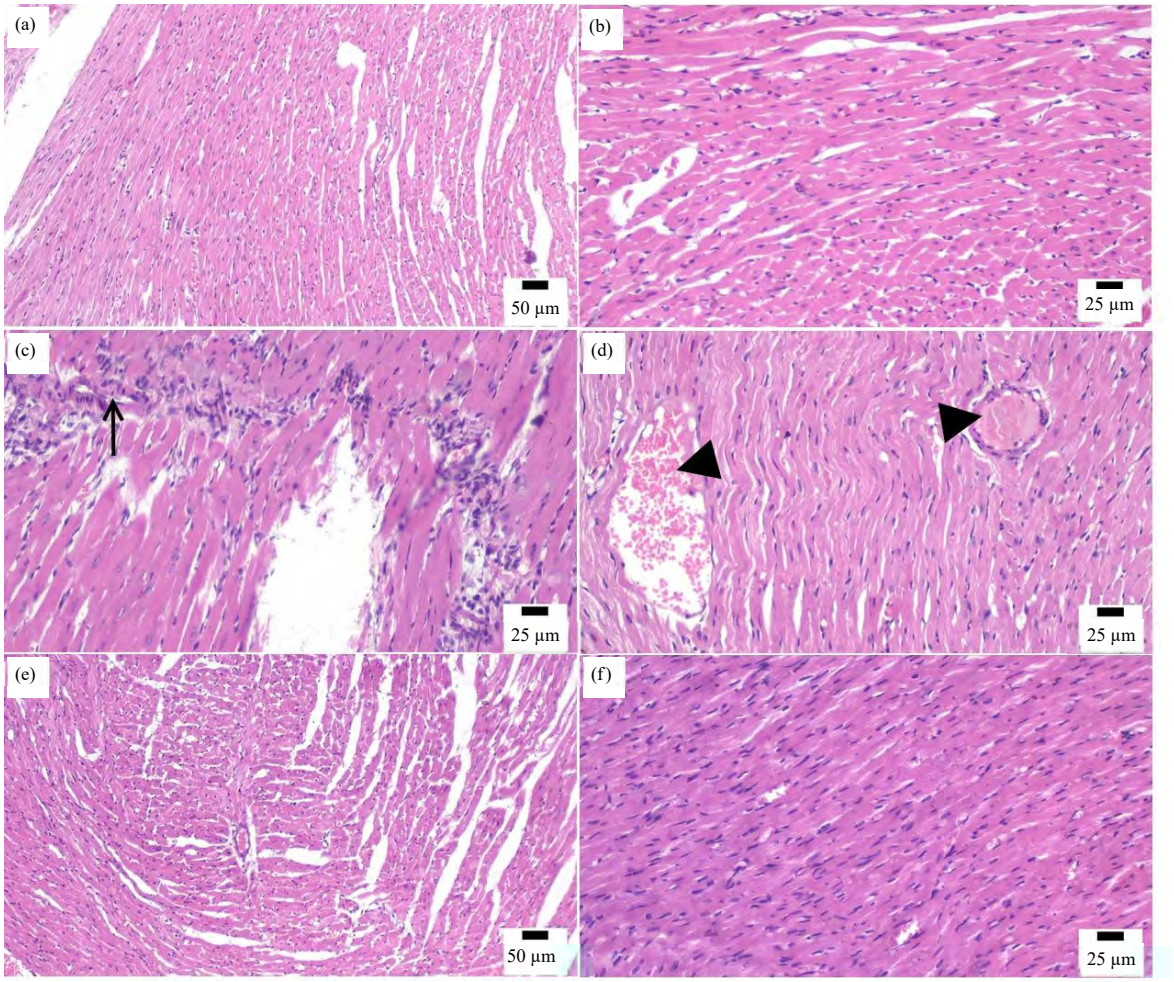

Fig. 2(a-f): Photomicrograph of the heart, (a-b) Microscopic observation of heart sections in the control group, (c-d) Microscopic observation of heart sections in MI group and (e-f) Microscopic observation of heart sections in earthworm extract group (H&E)
Infiltration of mononuclear inflammatory cells (arrow), Congested blood arteries (arrowhead)

Oxidative stress markers: The MDA and NO levels in the MI group showed a significant increase ($p<0.05$), while GSH, CAT and GST decreased compared with the control group. The values of the aforementioned parameters returned to near-normal levels after treatment with earthworm extract (Table 5).

Histopathology of the heart: The endocardium, myocardium and epicardium had a normal histological structure in the control group. The myocardial had cardiomyocytes that were cylindrical and branched, with acidophilic sarcoplasm and centrally placed oval nuclei (Fig. 2a, b). Several histological abnormalities were seen in the cardiac muscles of the MI group. The cardiac myocytes revealed a multifocal region of fibrosis associated with myocarditis, which was characterised by the infiltration of mononuclear inflammatory cells (arrow). Cardiomyocyte dispersion was seen in several of the studied areas due to inflammatory oedema. Degenerated cardiomyocytes that were vacuolated and profoundly eosinophilic were commonly detected. Severely congested blood arteries (arrowhead) were observed (Fig. 2c, d). The Earthworm group showed normal histological structure in most examined sections except for a few sections that displayed edematous exudates with a fewer number of infiltrated inflammatory cells (Fig. 2e, f).

Histopathology of the liver: The livers from the control group had normal histologic structure, with hepatocytes grouped in typical lobular architecture with central veins (CV) surrounded by radiating hepatic cords. The portal triads had a typical histological structure, including hepatic artery, portal vein and bile duct branches (Fig. 3a, b). The MI group revealed many

Table 5: Effect of E. worm on oxidative stress biomarkers of myocardial infarction rats

		Experimental groups		
Parameters	Organs	Control	MI	E. worm
MDA (nmol g^{-1} tissue)	Kidney	2.94±0.19[a]	5.02±0.16[b]	3.35±0.21[a]
	Liver	3.16±0.13[b]	4.19±0.13[c]	2.15±0.10[a]
	Heart	2.06±0.19[a]	3.41±0.22[b]	1.55±0.10[a]
NO (µmol g^{-1} tissue)	Kidney	4519.64±301[a]	6968.47±308[c]	5434.30±300.76[b]
	Liver	1944.98±59.01[a]	2895.79±113.27[c]	2615.19±31.21[b]
	Heart	1615.20±106.95[a]	2497.16±102.20[c]	2225.55±12.30[b]
GSH (mg g^{-1} tissue)	Kidney	0.54±0.03[c]	0.32±0.02[a]	0.44±0.01[b]
	Liver	0.61±0.01[b]	0.36±0.03[a]	0.65±0.07[b]
	Heart	1.36±0.08[c]	0.91±0.01[a]	1.19±0.05[b]
GST (U g^{-1} tissue)	Kidney	0.64±0.08[b]	0.28±0.02[a]	0.40±0.02[a]
	Liver	2.09±0.15[c]	1.40±0.01[a]	1.74±0.06[b]
	Heart	0.32±0.01[c]	0.10±0.00[a]	0.22±0.04[b]
CAT (U g^{-1} tissue)	Kidney	181.30±5.82[c]	106.33±16.30[a]	147.44±3.88[b]
	Liver	183.45±2.48[b]	128.19±5.26[a]	168.69±8.18[b]
	Heart	137.14±1.37[c]	39.83±2.77[a]	52.98±1.07[b]

Values are means±SE (n = 6 per group), each value not sharing a common letter superscript is significantly different (p<0.05)

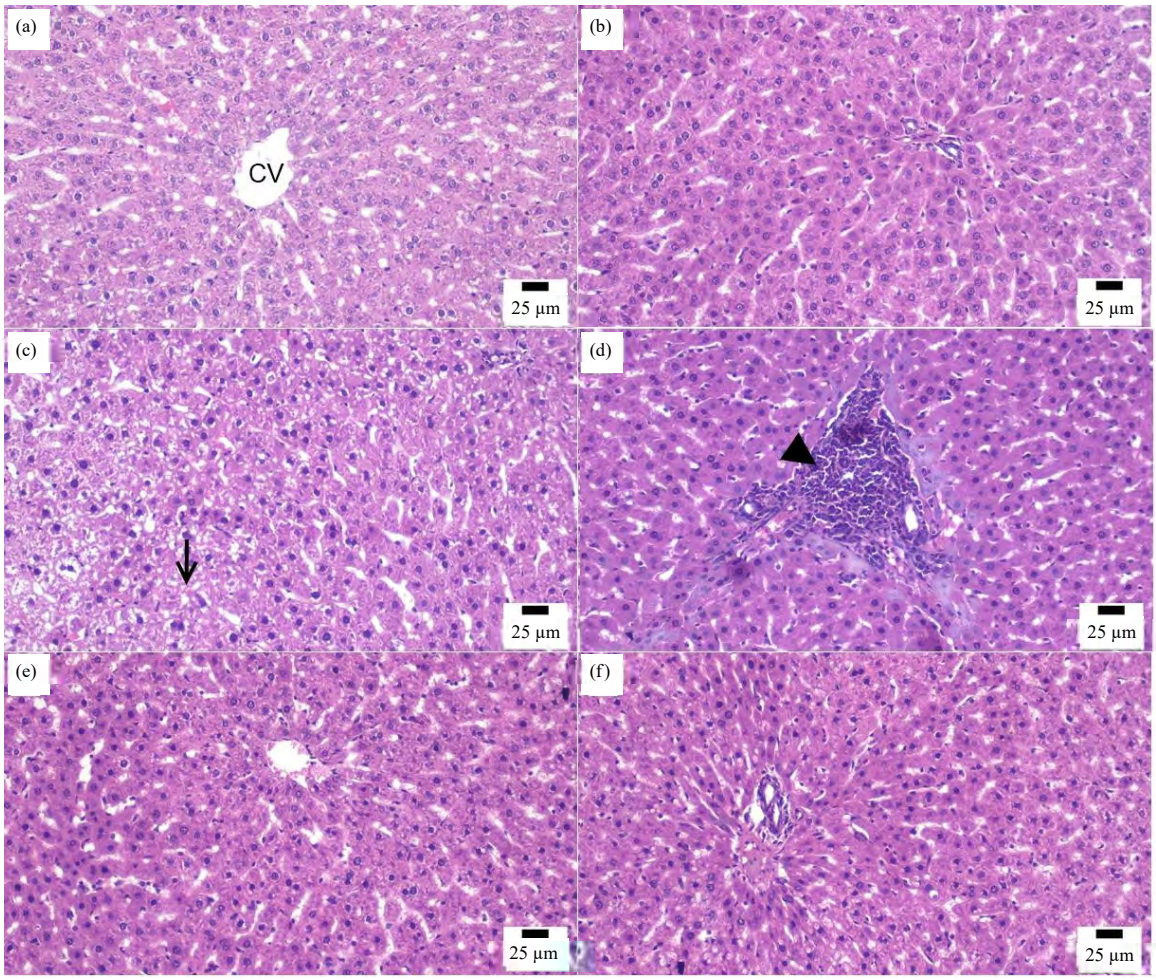

Fig. 3(a-f): Photomicrograph of the liver, (a-b) Microscopic observation of heart sections in the control group, (c-d) Microscopic observation of heart sections in MI group and (e-f) Microscopic observation of heart sections in earthworm extract group (H&E)
Central veins (CV), mononuclear inflammatory cells (arrowhead)

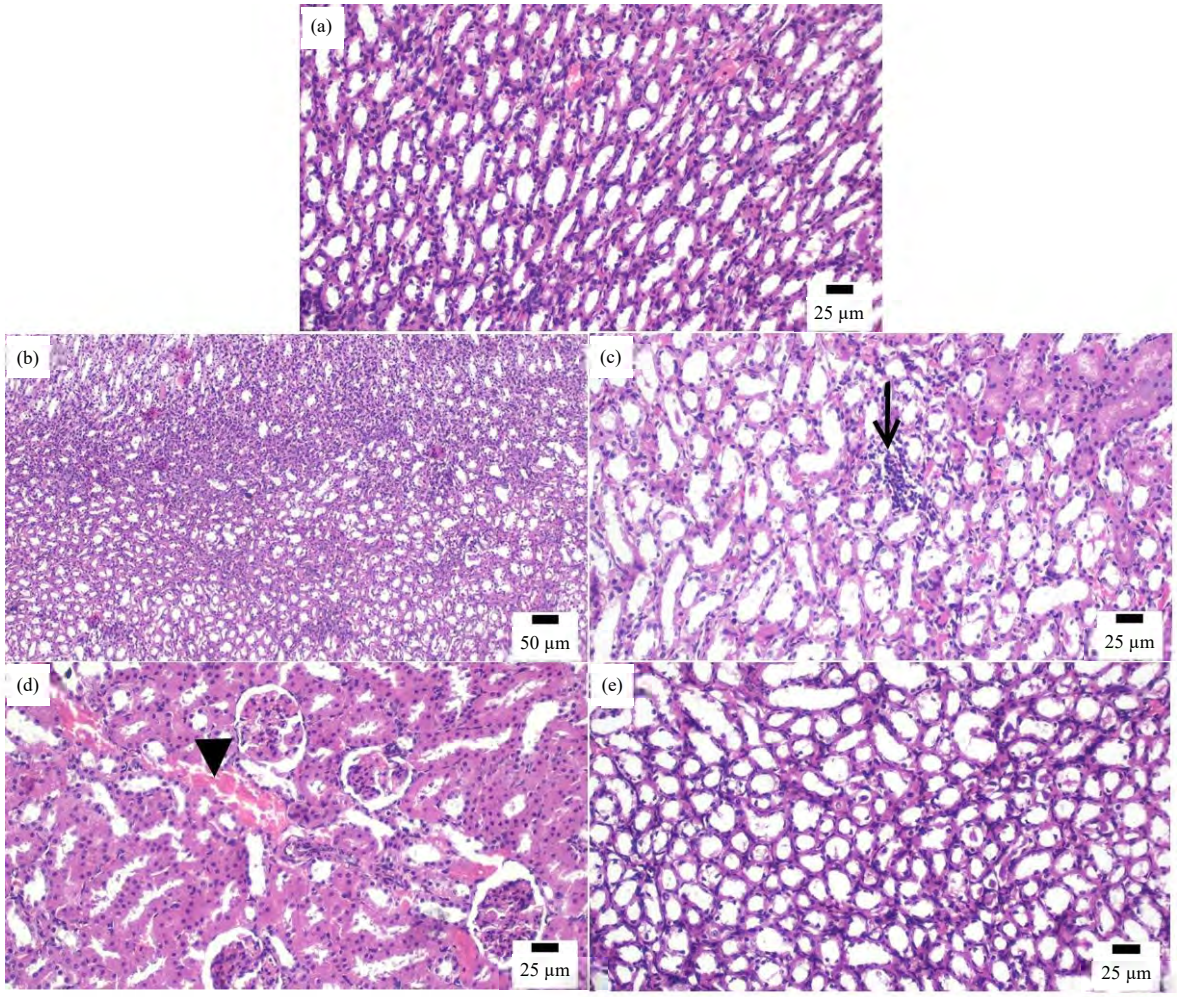

Fig. 4(a-f): Photomicrograph of the kidney, (a-b) Microscopic observation of heart sections in the control group, (c-d) Microscopic observation of heart sections in MI group and (e-f) Microscopic observation of heart sections in earthworm extract group (H&E)
Inflammatory cells infiltration (arrow), hyperemia (arrowhead)

histological changes in the investigated hepatic tissue. Random hepatic necrosis was observed in the hepatic lobules, which was coupled with necrobiotic alterations in the hepatocytes. In several of the investigated sections, the portal region revealed oedema associated with fibroplasia. In the hepatic parenchyma, multifocal aggregations of mononuclear inflammatory cells (arrowhead) were seen (Fig. 3c, d). Improvement of hepatic tissue was detected in rats who received earthworm.

However, few sections showed portal oedema associated with few inflammatory foci in the hepatic lobules (Fig. 3e, f).

Histopathology of the kidney: Microscopic investigation of kidney tissue from the control group (Fig. 4a) indicated normal histology of both the renal cortex and the medulla, the renal cortex included both glomeruli and renal tubules. The renal medulla had both kinds of tubules and collecting ducts. Examining the MI group (Fig. 4b, c) exhibited significantly congested renal parenchyma that was visible in the renal cortex, followed by dilated renal blood vessels and the formation of eosinophilic casts in the tubular lumen. Some examined medullary regions showed numerous inflammatory cells infiltration between renal tubules (arrow). Concerning the earthworm group (Fig. 4d, e) apparently normal kidney cytoarchitecture in numerous examined sections. Few sections showed mild hyperemia in the renal cortex (arrowhead) with few detectable renal casts and mildly dilated tubules in the medullary tubules.

DISCUSSION

Cardiovascular disorders are linked to high rates of morbidity and death worldwide[22]. Myocardial infarction (MI) is caused by an epinephrine overdose, which produces coronary vasoconstriction, raises myocardial oxygen demand and reduces myocardial blood flow[23].

In the current study, myocardial infarcted rats showed elevation in ST-segment in EEG and increases in the levels of cardiac troponin T (cTnT), creatinine kinase (CK) and lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Abnormalities on an ECG are the most common criterion for a definitive diagnosis of myocardial infarction[24]. These changes might be caused by oxidative stress that results in loss of cell membrane in the damaged heart[25,26]. Furthermore, myocardial creatine kinase leakage has been linked to ST elevation[27]. Epinephrine can also cause cardiac necrosis and expedite myocardial cell death[28], as seen in the current study's heart histology of MI- mice. These ECG changes are substantiated by the fact that epinephrine injection resulted in a significant increase in cardiac troponin T (cTnT), creatinine kinase (CK) and lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), which are markers of cardiac necrosis[29].

The cTnT, a well-established cardiomyocyte damage biomarker, is the chosen biomarker for acute MI diagnosis[30]. Although the mechanism of troponin release is unknown, it has been proposed that catecholamine-induced oxidative stress causes beta-adrenoreceptor hyperactivation, which leads to complicated functional, structural myocardial injury and permanent cellular damage and results in a release of cell proteins into the bloodstream[8,31].

The LDH, AST and CK, which are diagnostic indicators of cardiac tissue damage, leak out of injured tissues into the bloodstream when the cell membrane becomes permeable or ruptures. Changes in plasma membrane integrity and/or permeability are reflected in the quantity of these cellular enzymes present in plasma[32,33,26].

The current study showed that earthworm extract had cardioprotective properties, confirming normal ST-segment, restoring heart function to normal levels and protecting cardiac tissue from harm caused by a high dosage of Epinephrine.

The current investigation found a relation between MI and the liver by the significant increase in alanine amino transaminases (ALT), alkaline phosphates (ALP) and a reduction in total protein (TP) and albumin levels. In addition, histopathology analysis of liver tissue in the MI group demonstrated histological damage. The liver is a vital organ implicated in cellular redox equilibrium due to the synthesis of different intracellular antioxidant enzymes responsible for ROS clearance[34]. Clinical studies have demonstrated that hepatic necrosis can occur following an acute myocardial infarction[35,36]. Previous research has indicated that epinephrine-induced MI rats had reduced enzymatic free radical defence mechanisms, leading to increased oxidative stress that can contribute to pathological liver damage[37].

The earthworm extract treated rats showed improvement in liver function markers and histology. Suppresses the creation of reactive oxygen groups or scavenges them while concurrently modulating the genes responsible for antioxidant enzyme synthesis[16], inflammation suppression[38], cell regeneration acceleration and hepatic cell secretory processes enhancement are the hepatoprotective mechanisms of earthworm extract[39].

Current findings suggested that the kidney's capacity to operate had deteriorated, as demonstrated by the significant increase in creatinine, urea and uric acid and histopathological investigation. One of the fundamental MI caused by i.p., epinephrine injection is renal complication[40]. The pathogenetic mechanism of epinephrine-induced acute renal failure has been linked to ischemia insult, which contributes to the maintenance of renal dysfunction[40,41].

The renoprotective action of earthworm extract was discovered for the first time in this study, which was validated by restoring kidney function markers to normal levels and histological examination of renal tissue.

A high dosage of epinephrine produced oxidative stress in MI rats' hearts, livers and kidneys, as evidenced by large increases in MDA and NO levels and significant decreases in GSH, CAT and GST levels. The administration of high doses of epinephrine has been shown to cause significant oxidative stress and necrotic lesions in various organs[42]. Quinone metabolites of epinephrine can cause oxidative stress by reacting with oxygen to form ROS and interfering with antioxidant enzymes[43,44].

One of the most significant oxidative mechanisms that lead to cardiac cell damage is the conversion of Epinephrine to adrenochrome by superoxide anions[45,46]. Adrenochrome ($C_9H_9NO_3$) is a poisonous ortho-quinone molecule that binds with the sulphydryl groups of numerous proteins, including enzymes and reacts with oxygen to produce O^{2-} and H_2O_2 are part of its essential characteristics[47].

Earthworm extract showed potent antioxidant activity against MI-induced oxidative stress. It is a phenolic compound with anti-inflammatory[48] and antioxidant properties[49]. Because phenols contain hydroxyl groups, they are particularly effective for scavenging free radicals[50]. Since the earthworm's polyphenol content is high, the earthworm's anti-inflammatory and antioxidative properties may be linked to it[41].

These results showed the therapeutic ability of earthworm extract in the treatment of myocardial infarction, which opens the area to use this extract in clinical studies. More advanced studies on earthworm extract could be needed using many doses and periods before the clinical applications.

CONCLUSION

It may be concluded from the findings of this study that earthworm extract has therapeutic for myocardial infarction. Earthworm extract improves cardiac, liver and renal biomarkers and restores the EEG pattern to its original state. The therapeutic effect of earthworm extract results from its antioxidant and anti-inflammatory activities.

SIGNIFICANCE STATEMENT

This study discovers the therapeutic effect of earthworm extract for the treatment of myocardial infarction can be beneficial for the treatment of heart diseases. This study will help the researcher to uncover the critical areas of using natural products isolated from earthworms in medicine that many researchers were not able to explore. Thus a new theory on the uses of earthworm natural products in medicine may be arrived at.

REFERENCES

1. Xia, J.Y., D.M. Lloyd-Jones and S.S. Khan, 2019. Association of body mass index with mortality in cardiovascular disease: New insights into the obesity paradox from multiple perspectives. Trends Cardiovasc. Med., 29: 220-225.
2. Gaziano, T., K.S. Reddy, F. Paccaud, S. Horton and V. Chaturvedi, 2006. Cardiovascular Disease. In: Disease Control Priorities in Developing Countries, Jamison, D.T., J.G. Breman, A.R. Measham, G. Alleyne and M. Claeson *et al.* (Eds.), Oxford University Press, New York, pp: 645-662.
3. Liu, G., Y. Li, Y. Hu, G. Zong and S. Li *et al.*, 2018. Influence of lifestyle on incident cardiovascular disease and mortality in patients with diabetes mellitus. J. Am. Coll. Cardiol., 71: 2867-2876.
4. Bęćkowski, M., 2015. Acute coronary syndromes in young women-the scale of the problem and the associated risks. Kardiochirurgia Torakochirurgia, 12: 134-138.
5. Ugwu, C.E., S.E. Nwankwo, S.C. Meludu and J.K. Nnodim, 2016. Assessment of the risk of myocardial infarction among undergraduate students in a Nigerian tertiary institution. Int. J. Healthcare Med. Sci., 2: 60-65.
6. French, J., D. Brieger, C. Juergens, B. Costa, B. Carr, D.P. Chew and T. Briffa, 2020. Troponin measurements, myocardial infarction diagnoses and outcomes: An analysis of linked data from New South Wales, Australia. Internal Med. J., 50: 550-555.
7. Kumar, V.D.R. and K. Gurusamy, 2014. Antioxidant effect of *Garcinia indica* Linn fruit extract against isoprenaline hydrochloride induced myocardial necrosis in rats. Int. J. Pharm. Sci. Drug Res., 6: 220-223.
8. Moris, D., M. Spartalis, E. Spartalis, G.S. Karachaliou and G.I. Karaolanis *et al.*, 2017. The role of reactive oxygen species in the pathophysiology of cardiovascular diseases and the clinical significance of myocardial redox. Ann. Transl. Med., Vol. 5. 10.21037/atm.2017.06.27.
9. Liu, C., B. Li, Q. Yan, S. Niu and Y. Zhao *et al.*, 2021. Protective effects and mechanisms of recombinant human glutathione peroxidase 4 on isoproterenol-induced myocardial ischemia injury. Oxid. Med. Cell. Longevity, Vol. 2021. 10.1155/2021/6632813.
10. Cooper, J.A., J.D. Cooper and J.M. Cooper, 2006. Cardiopulmonary resuscitation: History, current practice and future direction. Circulation, 114: 2839-2849.
11. Wright, R.B., W.J. Pomerantz and J.W. Luria, 2002. New approaches to respiratory infections in children: Bronchiolitis and croup. Emergency Med. Clin. N. Am., 20: 93-114.
12. Radaković, M., S. Borozan, N. Djelić, S. Ivanović and D.Ć. Miladinović *et al.*, 2018. Nitroso-oxidative stress, acute phase response and cytogenetic damage in wistar rats treated with adrenaline. Oxid. Med. Cell. Longevity, Vol. 2018. 10.1155/2018/1805354.
13. Frishman, W.H., P. Beravol and C. Carosella, 2009. Alternative and complementary medicine for preventing and treating cardiovascular disease. Disease-a-Month, 55: 121-192.
14. Samatra, D.P.G.P., G.B.M. Tjokorda, I.D.M. Sukrama, N.W.S. Dewi, R.K. Praja, D. Nurmansyah and I.P.E. Widyadharma, 2017. Extract of earthworms (*Lumbricus rubellus*) reduced malondialdehyde and 8-hydroxy-deoxyguanosine level in male wistar rats infected by *Salmonella typhi*. Biomed. Pharmacol. J., 10: 1765-1771.
15. Prakash, M. and G. Gunasekaran, 2010. Gastroprotective effect of earthworm paste (*Lampito mauritii*, Kinberg) on experimental gastric ulcer in rats. Eur. Rev. Med. Pharmacol. Sci., 14: 171-176.
16. Balamurugan, M., K. Parthasarathi, L.S. Ranganathan and E.L. Cooper, 2008. Hypothetical mode of action of earthworm extract with hepatoprotective and antioxidant properties. J. Zhejiang Univ. Sci. B, 9: 141-147.

17. Balamurugan, M., K. Parthasarathi, E.L. Cooper and L.S. Ranganathan, 2009. Anti-inflammatory and anti-pyretic activities of earthworm extract-*Lampito mauritii* (Kinberg). J. Ethnopharmacol., 121: 330-332.
18. Chang, Y.M., Y.T. Shih, Y.S. Chen, C.L. Liu and W.K. Fang *et al*., 2011. Schwann cell migration induced by earthworm extract via activation of PAs and MMP2/9 mediated through ERK1/2 and p38. Evidence-Based Complementary Altern. Med., Vol. 2011. 10.1093/ecam/nep131.
19. Dewi, N.W.S., A.N. Mahendra, G.W.K. Putra, I.M. Jawi, D.M. Sukrama and N.L. Kartini, 2017. Ethanolic extract of the powder of red earthworm (*Lumbricus rubellus*) obtained from several organic farmlands in Bali, Indonesia: Analysis of total phenolic content and antioxidant capacity. Bali Med. J., 3: S80-S83.
20. Chinedu, E., D. Arome and F.S. Ameh, 2013. A new method for determining acute toxicity in animal models. Toxicol. Int., 20: 224-226.
21. Amin, M.M., O.N. El Gazayerly, N.A.A. El-Gawad, S.M.A. El-Halim and S.A. El-Awdan, 2016. Effect of formulation variables on design, *in vitro* evaluation of valsartan SNEDDS and estimation of its antioxidant effect in adrenaline-induced acute myocardial infarction in rats. Pharm. Dev. Technol., 21: 909-920.
22. Rizzetti, D.A., Á. Martín, P. Corrales, F. Fernandez, M.R. Simões *et al*., 2017. Egg white-derived peptides prevent cardiovascular disorders induced by mercury in rats: Role of angiotensin-converting enzyme (ACE) and NADPH oxidase. Toxicol. Lett., 281: 158-174.
23. El-Gohary, O.A. and M.M. Allam, 2017. Effect of vitamin D on isoprenaline-induced myocardial infarction in rats: Possible role of peroxisome proliferator-activated receptor-γ. Can. J. Physiol. Pharmacol., 95: 641-646.
24. Peacock, W.F., J.E. Hollander, R.W. Smalling and M.J. Bresler, 2007. Reperfusion strategies in the emergency treatment of ST-segment elevation myocardial infarction. Am. J. Emergency Med., 25: 353-366.
25. Münzel, T., G.G. Camici, C. Maack, N.R. Bonetti, V. Fuster and J.C. Kovacic, 2017. Impact of oxidative stress on the heart and vasculature: Part 2 of a 3- part series. J. Am. Coll. Cardiol., 70: 212-229.
26. Zhou, R., Q. Xu, P. Zheng, L. Yan, J. Zheng and G. Dai, 2008. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. Eur. J. Pharmacol., 586: 244-250.
27. Nabofa, W.E.E., O.O. Alashe, O.T. Oyeyemi, A.F. Attah and A.A. Oyagbemi *et al*., 2018. Cardioprotective effects of curcumin-nisin based poly lactic acid nanoparticle on myocardial infarction in guinea pigs. Sci. Rep., Vol. 8. 10.1038/s41598-018-35145-5.
28. Katrukha, I.A. and A.G. Katrukha, 2020. Myocardial injury and the release of troponins I and T in the blood of patients. Clin. Chem., 67: 124-130.
29. Chan, D. and L.L. Ng, 2010. Biomarkers in acute myocardial infarction. BMC Med., Vol. 8. 10.1186/1741-7015-8-34.
30. Laugaudin, G., N. Kuster, A. Petiton, F. Leclercq and R. Gervasoni *et al*., 2016. Kinetics of high-sensitivity cardiac troponin T and I differ in patients with ST-segment elevation myocardial infarction treated by primary coronary intervention. Eur. Heart J. Acute Cardiovasc. Care, 5: 354-363.
31. Vallipriya, R. and M.S. Begum, 2020. Cardio-protective effects of *Ipomea biloba* against the myocardial infarction in rats. Int. J. Pharm. Phytopharmacological Res., 10: 74-81.
32. Farvin, K.H.S., R. Anandan, S.H.S. Kumar, K.S. Shiny, T.V. Sankar and T.K. Thankappan, 2004. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. Pharmacol. Res., 50: 231-236.
33. Gürgün, C., M. Ildizli, O. Yavuzgil, A. Sin, A. Apaydin, C. Çinar and H. Kültürsay, 2008. The effects of short term statin treatment on left ventricular function and inflammatory markers in patients with chronic heart failure. Int. J. Cardiol., 123: 102-107.
34. Birrer, R., Y. Takuda and T. Takara, 2007. Hypoxic hepatopathy: Pathophysiology and prognosis. Internal Med., 46: 1063-1070.
35. Butler, D.C., D.N. Lewin and N.I. Batalis, 2018. Differential diagnosis of hepatic necrosis encountered at autopsy. Acad. Forensic Pathol., 8: 256-295.
36. Oh, P.C., Y.S. Eom, J. Moon, H.J. Jang and T.H. Kim *et al*., 2020. Prognostic impact of the combination of serum transaminase and alkaline phosphatase determined in the emergency room in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. PLoS ONE, Vol. 15. 10.1371/journal.pone.0233286.
37. Melekh, B., I. Ilkiv, A. Lozynskyi and A. Sklyarov, 2017. Antioxidant enzyme activity and lipid peroxidation in rat liver exposed to celecoxib and lansoprazole under epinephrine-induced stress. J. Appl. Pharm. Sci., 7: 94-99.
38. Dajem, S.B., S.B. Ali, O.G. Abdelrady, N.M. Salahaldin and A.M. Soliman *et al*., 2020. *Allolobophora caliginosa* coelomic fluid ameliorates gentamicin-induced hepatorenal toxicity in rats. Asian Pac. J. Trop. Biomed., 10: 411-416.
39. Balamurugan, M., 2007. Restoration of histoarchitecture in the paracetamol-induced liver damaged rat by earthworm extract, *Lampito mauritii* (Kinberg). Eur. Rev. Med. Pharmacol. Sci., 11: 407-411.
40. Salama, A., D. Mansour and R. Hegazy, 2021. The cardio and renoprotective role of ginseng against epinephrine-induced myocardial infarction in rats: Involvement of angiotensin II type 1 receptor/protein kinase C. Toxicol. Rep., 8: 908-919.

41. Balamurugan, M., K. Parthsarathi, E.L. Cooper and L.S. Ranganathan, 2007. Earthworm paste (*Lampito mauritii* Kinberg) alters inflammatory, oxidative, haematological and serum biochemical indices of inflamed rat. Eur. Rev. Med. Pharmacol. Sci., 11: 77-90.
42. Korać, J., D.M. Stanković, M. Stanić, D. Bajuk-Bogdanović and M. Žižić *et al*., 2018. Coordinate and redox interactions of epinephrine with ferric and ferrous iron at physiological pH. Sci. Rep., Vol. 8. 10.1038/s41598-018-21940-7.
43. Costa, V.M., R. Silva, L.M. Ferreira, P.S. Branco and F. Carvalho *et al*., 2007. Oxidation process of adrenaline in freshly isolated rat cardiomyocytes: Formation of adrenochrome, quinoproteins and GSH adduct. Chem. Res. Toxicol., 20: 1183-1191.
44. Senthil, S., M. Sridevi and K.V. Pugalendi, 2007. Cardioprotective effect of oleanolic acid on isoproterenol-induced myocardial ischemia in rats. Toxicol. Pathol., 35: 418-423.
45. Dhalla, N.S., M.R. Dent and A.S. Arneja, 2008. Pathogenesis of Catecholamine-Induced Cardiomyopathy. In: Cardiovascular Toxicology, Acosta, D. (Ed.), CRC Press, United States, pp: 207-262.
46. Dhalla, N.S., A. Adameova and M. Kaur, 2010. Role of catecholamine oxidation in sudden cardiac death. Fundam. Clin. Physiol., 24: 539-546.
47. Adameova, A., Y. Abdellatif and N.S. Dhalla, 2009. Role of the excessive amounts of circulating catecholamines and glucocorticoids in stress-induced heart disease. Can. J. Physiol. Pharmacol., 87: 493-514.
48. Calixto, J.B., M.M. Campos, M.F. Otuki and A.R.S. Santos, 2004. Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Med., 70: 93-103.
49. Yousse, A., A. Baiomy, S.R. Fahmy, A. Mohamed, D. Saad and R. Desoky, 2022. Potential anti-osteoporotic effect of *Allolobophora caliginosa* extract in orchiectomized rats. Pharm. Sci. Asia, 49: 138-146.
50. Mathew, S., T.E. Abraham and Z.A. Zakaria, 2015. Reactivity of phenolic compounds towards free radicals under *in vitro* conditions. J. Food Sci. Technol., 52: 5790-5798.