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CARDIOPROTECTIVE EFFECT OF METHANOL EXTRACT OF *JATROPHA TANJORENSIS* LEAVES IN ISOPRENALINE INDUCED MYOCARDIAL INFARCTION IN ALBINO RATS: CARDIAC FUNCTION BIOMARKERS, ANTIOXIDANT AND HEART HISTOARCHITECTURE EVALUATION

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ABSTRACT

Background of the Study: Coronary artery disease and its end results, myocardial infarction is a significant cause of morbidity and mortality globally. Recently, there is an increase in the incidence of myocardial infarction which manifest as a result of disrupted blood supply and oxygen to the myocardium. **Objective**: This present study evaluated the cardioprotective effect of methanol extract of *Jatropha tanjorensis* leaves on some cardiac functions biomarkers, endogenous antioxidant activity and pathology of the heart in isoprenaline induced myocardial infarction in albino rats. **Method**: Seventy two albino rats were randomly divided into six (6) groups of Twelve rats per group. Group 1 served as the normal control, group 2 was the negative control (administered 85mg/kg of isoprenaline only), group 3 served as the positive control (pretreated with 2mg/kg carvedilol for 28 days, group 4 through 6 were pretreated with 200mg/kg, 400mg/kg and 600mg/kg the extract respectively for 28 days. Myocardial infarction was induced in the rats using subcutaneous injection of 85mg/kg Isoprenaline (ISO) for two consecutive days (26^{th} and 27^{th}) at 24hours interval. **Results:** The result of the study showed the extract at the dose of 400mg/kg significantly ($p<0.05$) decrease CK-MB, Troponin I, LDH, hsCRP and MDA compared to the negative control. There was also

significant ($p<0.05$) increase in catalase, SOD, GPx and GSH of the treated groups except the group administered 600mg/kg of the extract which showed significant decrease in the catalase level compared to normal and other groups. **Conclusion**: The extract at 400mg/kg dose showed better potency in preventing the damage of cardiomyocytes though there was mild alteration in the heart histoarchitecture. This study suggested that the extract to some extent possess mild cardioprotective potency at certain dose range.

KEYWORDS: myocardial infarction, isoprenaline, cardiac biomarkers, carvedilol, *Jatropha tanjorensis.*

INTRODUCTION

Globally, there is increase in the number of death resulting from the generation of free radicals and oxidative stress. Metabolic pathway and environmental pollution easily generate free radicals which are also regarded as reactive species (Souri *et al.*, 2008; Neha and Lubna, 2014). Different factors can elevate and accumulate the level of oxidative stress and free radicals which easily prevent the cell from working effectively. This can then lead to damage of cells, and has main contributory role in pathogenesis of cardiovascular diseases (Mohanty *et al.*, 2007; Mahammad *et al.*, 2012).

According to the American Heart Association and World Health Organization statistics, cardiovascular diseases (CVDs) are regarded as the main cause of death worldwide (Mozaffarian *et al*., 2016). CVD mostly refers to MI (Myocardial infarction), angina pectoris, hypertension, stroke and other circulatory diseases. Coronary artery diseases, congestive heart failure, cardiac arrest, arrhythmias, and peripheral artery diseases are the most commonly reported heart diseases (Mallapu *et al*., 2017).

A coronary artery disease which is also called Ischemic heart disease is a crucial problem worldwide and it's known as major non transmissible disease (Abubaker *et al*., 2012). A good example of ischemic heart disease is acute myocardial infarction (MI) and it manifest due to inequality between coronary blood supply and myocardial demand. Myocardial damage due to free radicles is an imperative etiological mechanism that is linked with increased level of reactive oxygen species and/or insufficient antioxidant defense system (Kharadi *et al*., 2016). Isoprenaline (ISO) is synthetic catecholamines with active effect on non-selective beta-adrenergic agonist and low affinity for alpha adrenergic receptors. It has the tendency to produce myocardial cell death in high dose. Various drugs with

cardioprotective effect have been studied using isoprenaline induced myocardial infarction model. Administration of isoprenaline in high doses can lead to myocardial lesions. The various adverse effect associated with modern medicines has limited their effective used in preventing heart diseases (Upaganlawar *et al*., 2011).

Leafy vegetable play vital parts in the food culture of many African households and it is part of Africans' cultural heritage (Mensah *et al.*, 2008; Omoregie *et al.*, 2011). Nigeria is blessed with a multiplicity of indigenous green leafy vegetables which are consumed by various groups for different purpose. Medicinal plants are unique in their ability to treat several human ailments because they contained various valuable phytoconstituents. Secondary plant metabolites such as steroids, alkaloids, flavonoids, glycosides, terpenoids, tannins, saponins, phenolic compounds etc. are mainly accountable for the healing potency of the plant (Omoregie *et al.*, 2011).

Medicinal plants have been used for centuries to combat many health challenges and are also useful component in pharmaceutical industries; among these plants is *Jatropha tanjorensis. Jatropha tanjorensis* known as Chaya leave and generally known as 'Hospital too far' in Nigeria is a shrub from the family Euphorbiaceae. Different parts of *Jatropha* plants are used in many ways and in different countries.

Jatropha tanjorensis leaves has been reported to possess numerous medicinal properties such as hepatoprotective (Ezeonu *et al.*, 2017; Madubuike *et al*., 2015), antidiabetes (Momoh *et al*., 2014; Chinenye *et al*., 2019), anticancer (Purshothaman *et al.*, 2014), antianaemic (Ameloko 2010; MacDonald *et al*., 2014), antiulcer (Epison *et al*., 2016), hypolipidemic (Oyewole and Akingbala, 2011; Ijioma *et al*., 2014), antioxidant (Omoregie *et al*., 2011), antibacterial (Oboh and Masodje, 2009; Daniyan *et al.*, 2018) among others. This study evaluated the effect of methanol extract of *Jatropha tanjorensis* leaves on some cardiac function biomarkers in isoprenaline induced myocardial infarction in albino rats.

MATERIALS AND METHODS

Collection of Plant Leave

Jatropha tanjorensis leaves were collected from the premises of Federal Polytechnic Nekede Owerri and identified by a Taxonomist, Dr G. Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The leave was washed with distilled water and dried for about seven days at room temperature.

The dried leaves were pulverized into fine powder using Pulverizer (5126 TP) and preserved in cellophane bags until when used.

Chemicals/reagents used

All the chemicals used in this study were of analytical grades and products.

Methanol was product of BDH Chemical Company Sule and Arhoghro 164 Ltd, Poole, England. Rat feed was purchased from Pfizer Nigeria Plc. Biochemical kits were products of Randox Diagnostics, Crumlin, UK. Carvedilol was product of Selleck chemicals, Germany.

Extract Preparation

Five hundred gram (500g) of powdered leave was macerated in 2.5L of methanol at room temperature for 72h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was dried in a water bath at 45ºC and concentrate was kept in air tight bottle at 4°C until used (Unegbu *et al.*, 2017).

Experimental animals

Seventy two (72) male albino rats of the Wistar strain aged 10 – 12 weeks and weighing 80 – 120 g and 18 mice weighing 16 – 22 g were procured from the Animal House, of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in standard transparent cages with wheat husk bedding, renewed every 24 hour. They were kept under controlled room temperature and humidity (25 to 29 °C; 30 to 70 %) in a 12 hour light-dark cycle. Animals were acclimatized for two weeks to laboratory conditions before starting the experiment. The animals were given standard rat feed (vital feeds with 18% crude protein and 2800kcal/kg metabolizable energy) and water *ad libitum*. Care of experimental animals was taken as per the guidelines given by NRC (2011) and the protocol was approved by Animal use Ethical Committee of Michael Okpara University of Agriculture Umudike with Ethical number BCM/EC/02/072.

Phytochemical Screening

The qualitative phytochemical screening was carried out using the methods of Harborne (1973) and Trease and Evans (1989).

Acute Toxicity (LD_{50})

The acute toxicity of the *Jatropha tanjorensis*leaves was determined using Lorke's method (1983).

Experimental Design

In a completely Randomized Design (CRD), the *Wistar* albino rats was allocated randomly into 6 treatment groups of 12 rats per group. Myocardial infarction was induced in rats by giving Isoprenaline (ISO) (85 mg/kg) subcutaneously (s.c.) for two subsequent days, on day 26 and 27 at the interval of 24 hours. Distribution of study groups was as follow.

Group 1 (normal control) rats were given distilled water orally for 28 days and normal saline s.c. on the day 26 and 27.

Group 2 (negative control) rats were given distilled water orally for 28 days and ISO (85 mg/kg) s.c. on the day 26 and 27.

Group 3 (positive control) rats were given carvedilol (2 mg/kg) orally for 28 days and ISO (85 mg/kg) s.c. on the day 26 and 27.

Group 4 rats were given *Jatropha tanjorensis* leaf extract 200 mg/kg orally for 28 days and ISO (85 mg/kg) s.c. on the day 26 and 27

Group 5 rats were given *Jatropha tanjorensis* leaf extract 400 mg/kg orally for 28 days and ISO (85 mg/kg) s.c. on the day 26 and 27.

Group 6 rats were given *Jatropha tanjorensis* leaf extract 600 mg/kg orally for 28 days and ISO (85 mg/kg) s.c. on the day 26 and 27.

At end of the experiment, the rats were sacrificed and blood samples for biochemical assays were collected in plain tubes and allowed to clot before centrifugation and the sera were separated thereafter and used for the assays. The heart was further harvested, fixed in 10 % buffered formalin and used for histopathological studies.

Biochemical estimations

Determination of CK-MB Activity

This was determined using Immuno inhibition method as used by Kharadi *et al. (*2016) and described by TIETZ, (1999) and Mattenheimer (1981).

Determination of High Sensitivity C-Reactive Protein (hsCRP)

The High Sensitivity C-Reactive Protein (hsCRP) was determined using a method described by Young (1995) and TIETZ (1999)

Determination of troponin level: Troponin was assayed using ELISA method.

Lactate Dehydrogenase (LDH) activity

LDH was measured by UV kinetic method as described by Henry 1979.

Evaluation of Antioxidant

Estimation of extent of lipid peroxidation

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.* (1993).

Assay of superoxide dismutase activity

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as adopted in Randox kit.

Assay for catalase activity: Catalase activity was assayed by the method of Sinha (1972).

Estimation of glutathione peroxidase activity: This was done according to the method described by Paglia and Valentine (1967).

Reduced glutathione estimation

The reduced glutathione level was determined by the method of Exner *et al.* (2000).

Histopathological examination

The method described by Sarowoot and Chuchard (2013) with slight modification was used. The experimental animals were euthanized at the end of the study period. Tissue sections of the liver from each group were collected for histopathological studies. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours prior to tissue preparation. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the tissue-containing wax blocks were cut into 5µm thick sections with a rotary microtome, floated in water bathe and incubated at 60˚C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant.

Slide Examination: The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at x100 and x400 magnifications.

Statistical analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The statistically analysed data were reported as Mean+SEM. Significant difference was accepted at 95% confidence level of probability ($P < 0.05$).

RESULTS

Table 1: Phytochemicals present in methanol extract of *Jatropha tanjorensis* leaves.

Phytochemicals	Specific Test	Inference
Phenols	Ferric chloride test	+
Flavonoids	Sodium Hydroxide test	++
Saponins	Frothing test	+
Alkaloids	Wagner's test	++
Tannins	Ferric chloride test	+
Terpenoids	Salkowski test	+
Steroids	Liebermann test	+

The phytochemical content of *Jatropha tanjorensis* revealed the bioactive compounds shown in the table above.

+ = present, ++ highly present.

Table 2: Acute Toxicity Study of methanol extract of *Jatropha tanjorensis* leaves.

Groups	Concentration (mg/kg)	Average weight of the animals (g)	Mortality/signs of toxicity	Number of animals that survived
Phase 1				
1	10	18	Nil	3/3
2	100	17	Nil	3/3
3	1000	20	Nil	3/3
Phase 2				
1	1600	16	Nil	3/3
2	2900	21	Nil	3/3
3	5000	22	No death but decrease in appetite	3/3

The result above showed that the *Jatropha tanjorensis* leaves extract does not have any acute toxicity since no mortality was recorded at the highest dose of 5000mg/kg though there was loss of appetite by the animal administered 5000mg/kg of *Jatropha tanjorensis.*

Table 3: Body weight, Organ weights and various organs to body ratio.

Groups	Body Weight (BW) (g)	Heart weight (HW) (g)	HW/BW (10^{-3})
Normal control	102.39±2.43	0.53±0,03	5.18±0.34
Negative control	122.81±5.00	0.87±0.04	7.08±0.21
Positive control	120.30±9.26	0.65±0.02	5.54±0.42
200mg/kg extract	139.23±6.22	0.69±0.04	4.97±0.18
400mg/kg extract	107.01±4.63	0.72±0.03	6.79±0.34
600mg/kg extract	133.26±7.36	0.79±0.06	5.82±0.15

The above showed the body weight and organ weight with the relative ratio. There is non-significant ($p>0.05$) changes in the groups administered 200mg/kg of extract compared to the 2mg/kg carvedilol (positive) control for heart to body ratio. There was slight significant ($p<0.05$) increase in heart to body ratio in the negative control.

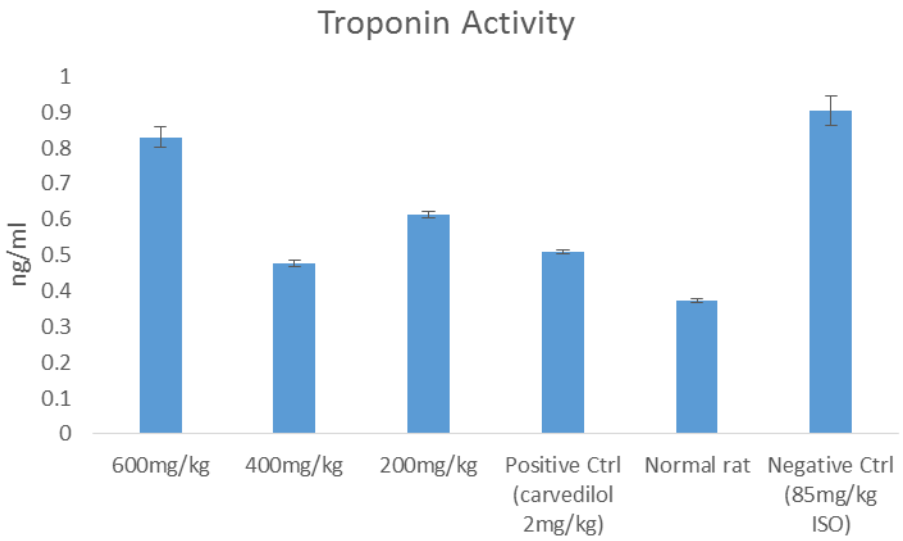


Figure 1: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum troponin activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows significant ($p<0.05$) decrease in the 400mg/kg and 200mg/kg extract treated group when compared with the negative control group. There was significant ($p<0.05$) increase in the group administered 600mg/kg of the extract when compared with the positive rats. The groups administered 400mg/kg of extract shows non-significant ($p>0.05$) increase when compared with the group treated with 2mg/kg carvedilol (positive control).

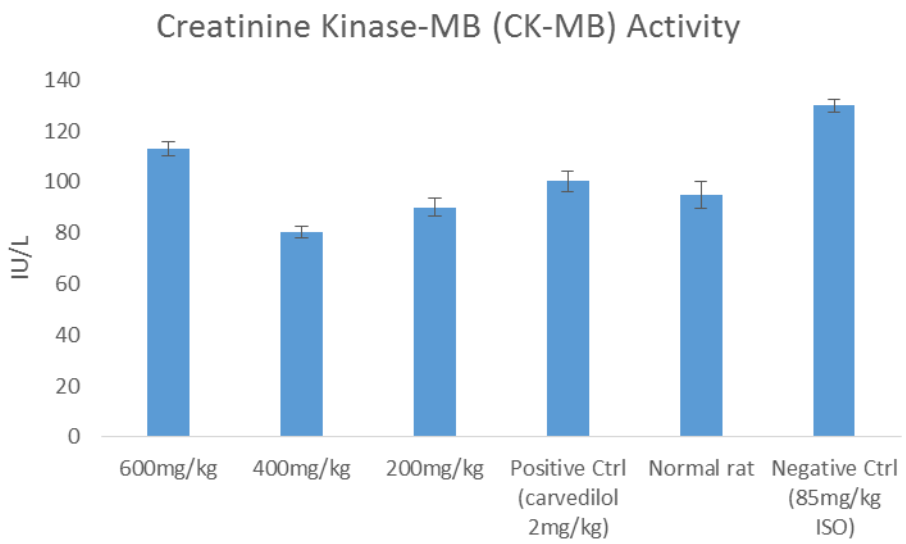


Figure 2: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum CK-MB activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows significant ($p<0.05$) decrease in the group treated with 400mg/kg and 200mg/kg extract when compared with the negative control group (85mg/kg ISO only). There was significant ($p<0.05$) increase in the group administered 600mg/kg of the extract when compared with the positive control.

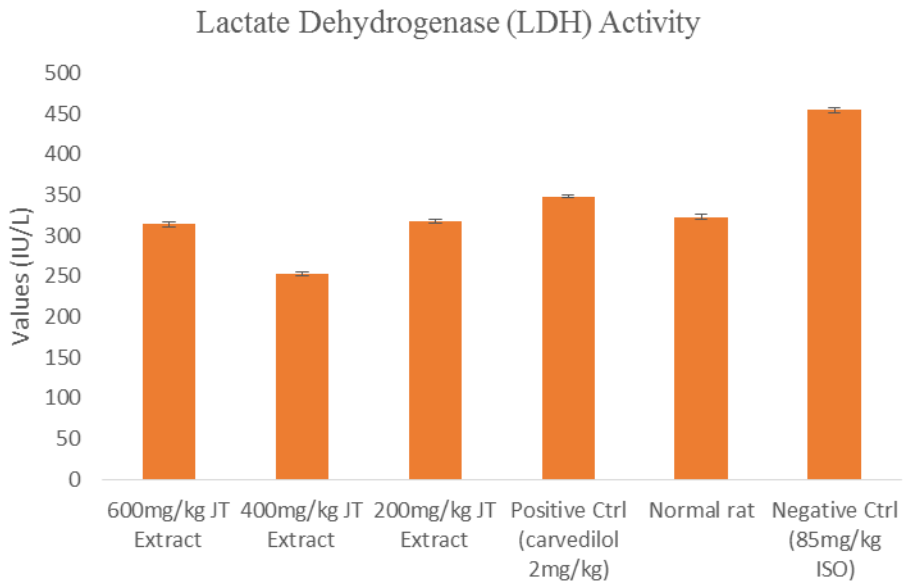


Figure 3: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum LDH activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows significant ($p<0.05$) decrease in the group treated with 400mg/kg extract when compared with all the control groups. There was also significant ($p<0.05$) decrease in the groups administered 200mg/kg and 600mg/kg of the extract when compared with the negative control.

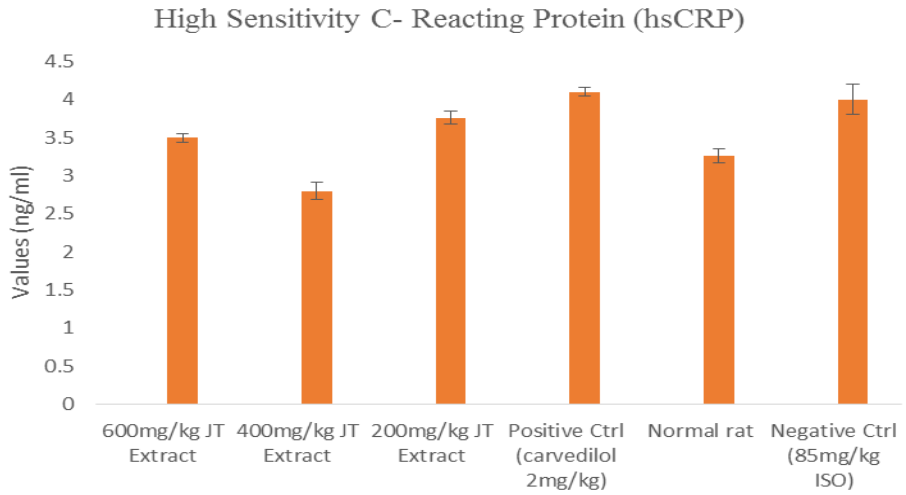


Figure 4: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum hsCRP activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows significant ($p<0.05$) decrease in the group treated with 400mg/kg of the extract when compared with the negative and positive control groups. There was non-significant ($p<0.05$) increase in the groups administered 200mg/kg and 600mg/kg of the extract when compared with the positive control.

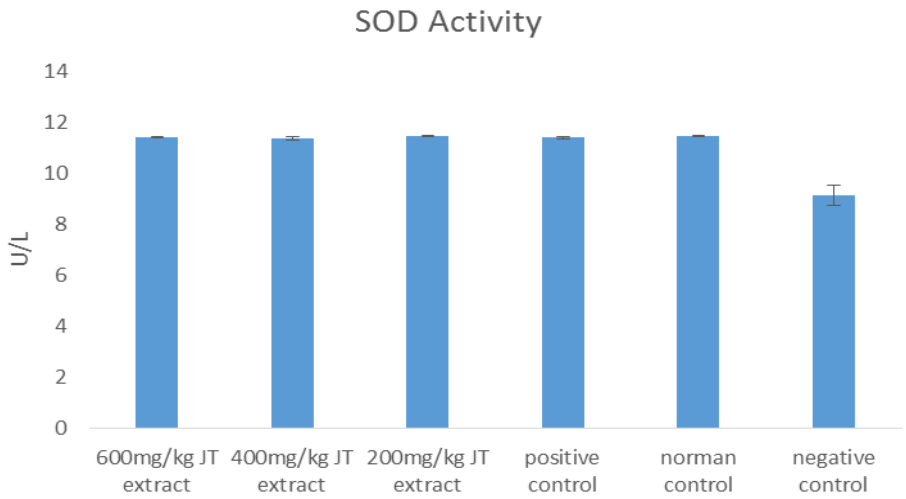


Figure 5: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum SOD activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows non-significant (p>0.05) decrease in the extract treated groups when compared with the positive and normal control. There was significant (p<0.05) increase in the extract treated groups when compared with the negative control.

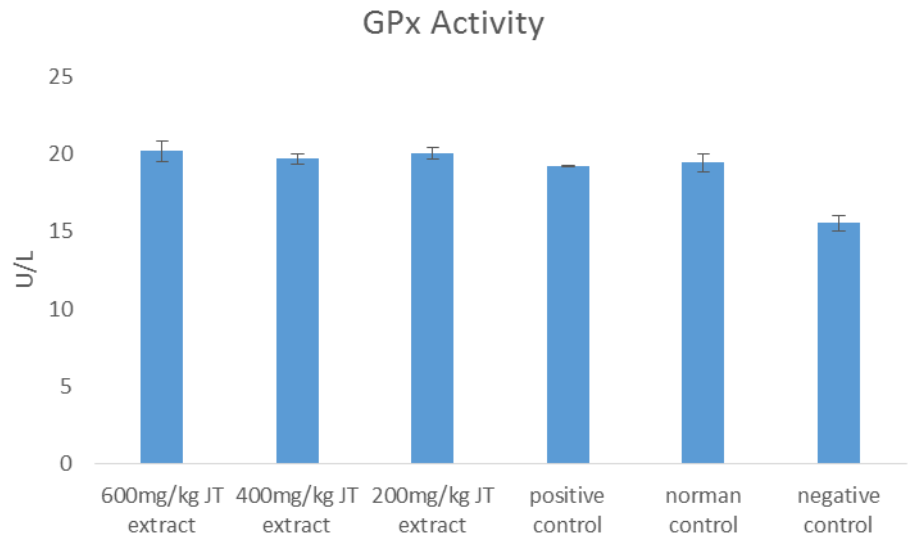


Figure 6: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum GPx activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows non-significant (p>0.05) decrease in the extract treated groups when compared with the positive and normal control. There was significant (p<0.05) increase in the extract treated groups when compared with the negative control.

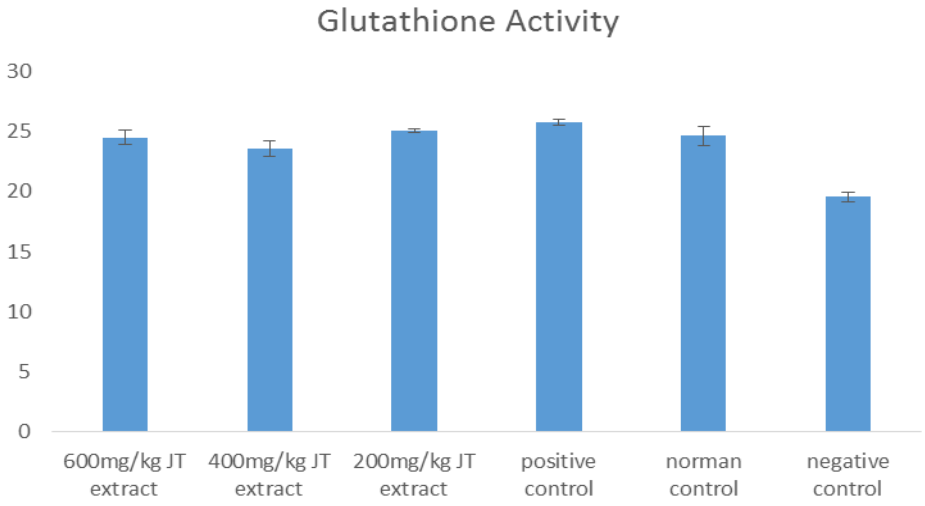


Figure 7: Result on the Effect of methanol leaf extract of *Jatropha tanjorensis* on serum glutathione concentration of isoprenaline induced myocardial infarction in albino rats.

The result above shows non-significant ($p>0.05$) decrease in the 200mg/kg, 400mg/kg and 600mg/kg extract treated groups when compared with the positive and normal control. There was significant ($p<0.05$) increase in the 200mg/kg, 400mg/kg and 600mg/kg extract treated groups when compared with the negative control.

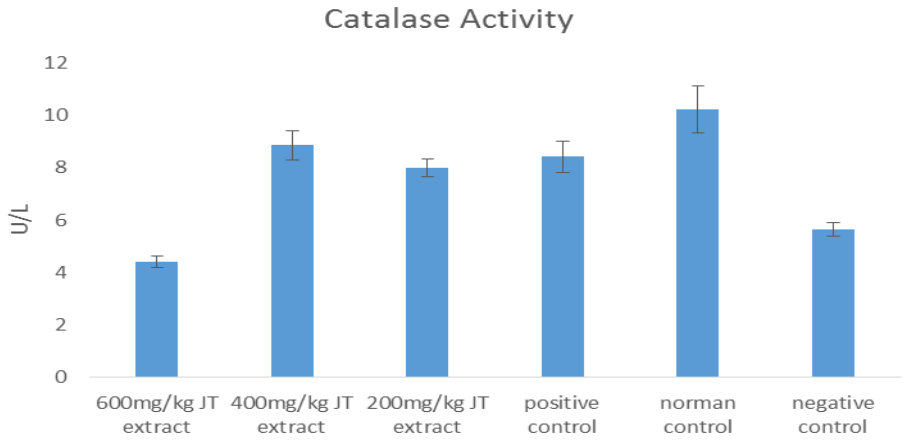


Figure 8: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum catalase activity of isoprenaline induced myocardial infarction in albino rats.

The result above shows significant ($p<0.05$) increase in the group administered with 200mg/kg and 400mg/kg of the extract when compare with the negative control. There was non-significant (>0.05) difference in the 400mg/kg extract treated group compared to positive control. There was significant ($p<0.05$) decrease in the group administered 600mg/kg extract when compared with the positive and normal control.

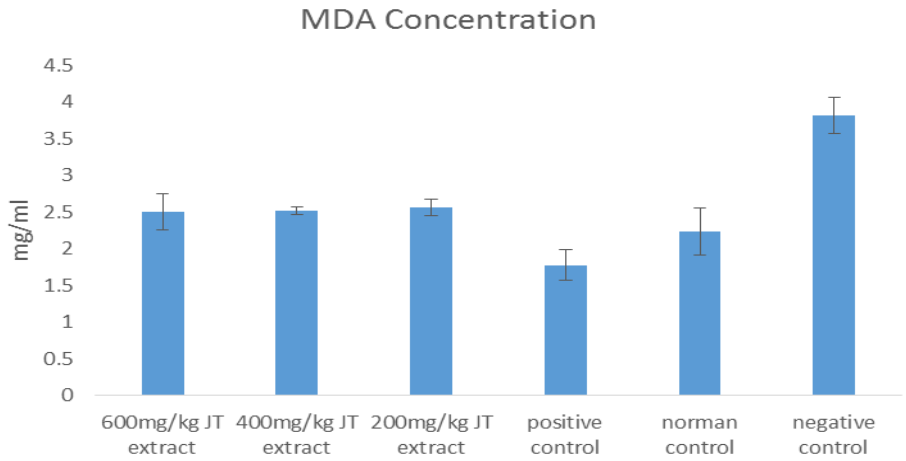


Figure 9: Result on the effect of methanol leaf extract of *Jatropha tanjorensis* on serum MDA concentration of isoprenaline induced myocardial infarction in albino rats.

The result above shows significant ($p<0.05$) decrease in the extract treated groups when compared with the negative control group. There was significant ($p<0.05$) difference in the extract treated groups when compared with positive control. But there was non-significant ($p>0.05$) increase in the extract treated groups when compared with the normal control.

Results on Histopathology of the heart, liver and kidney

The section of the heart collected from the negative control and group administered 600mg/kg of the extract showed histomorphological alteration, severe and mild widespread myocardial necrosis. The 600mg/kg group also showed multifocal area of myocardial cellular degeneration, swollen muscle cells with pale cytoplasm, loss of cross striations, fragmemtation and clumping of the myocardial cells when compared with the normal control.

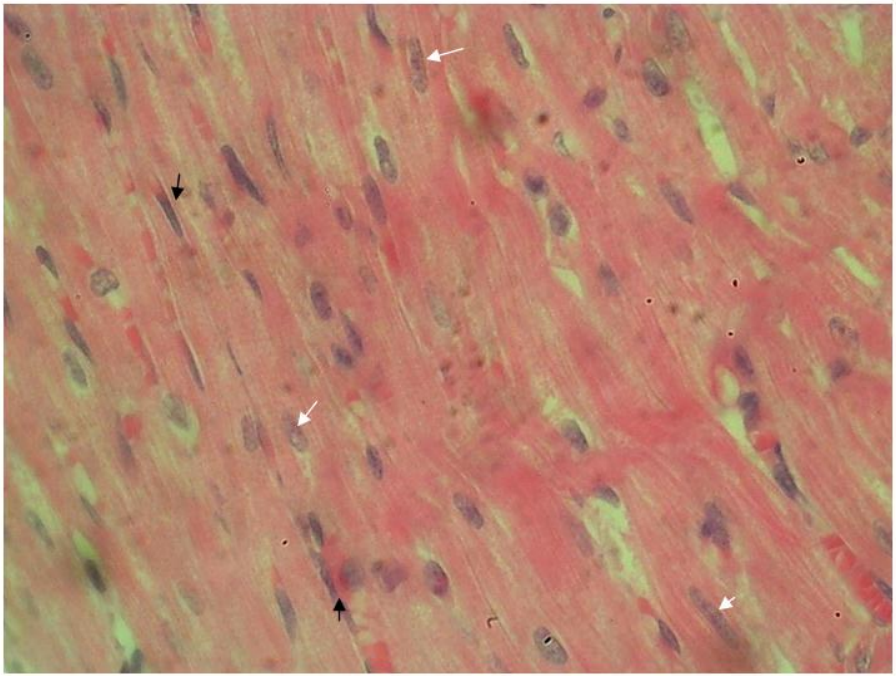


Figure 10: Sections of the heart collected from rats in Normal control group (normal rats) showed the normal myocardial histomorphology. Longitudinal section of the heart showing elongated nuclei of the myocardiocytes (white arrow) and pericytes (black arrow). H&E x400.

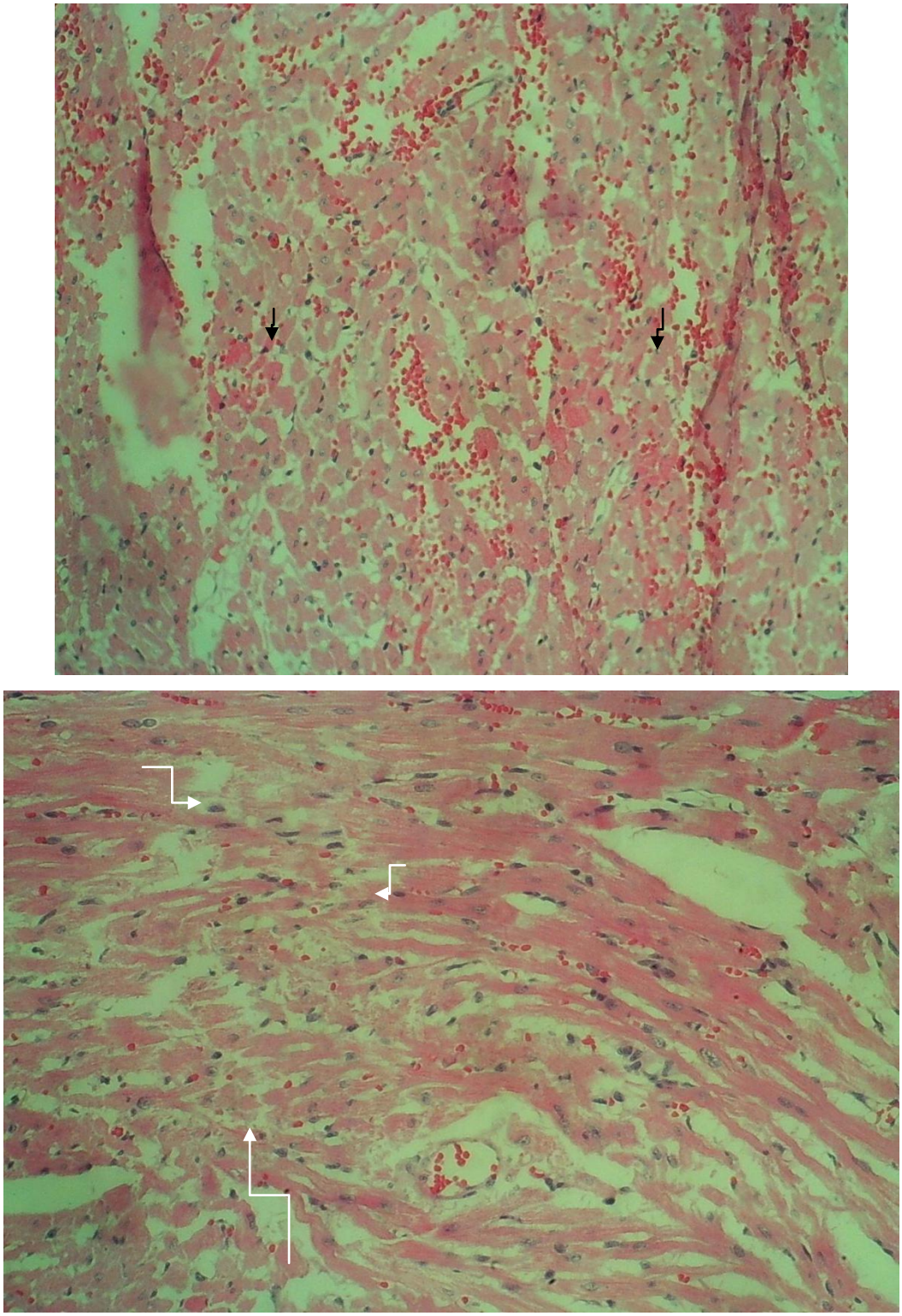


Figure 11: Sections of the heart collected from rats in Negative control group showed a severe widespread myocardial necrosis. Affected areas showed fragmentation and loss of striations (white arrow) admixed with typical Zenker's necrosis (black arrow). H&E x16.

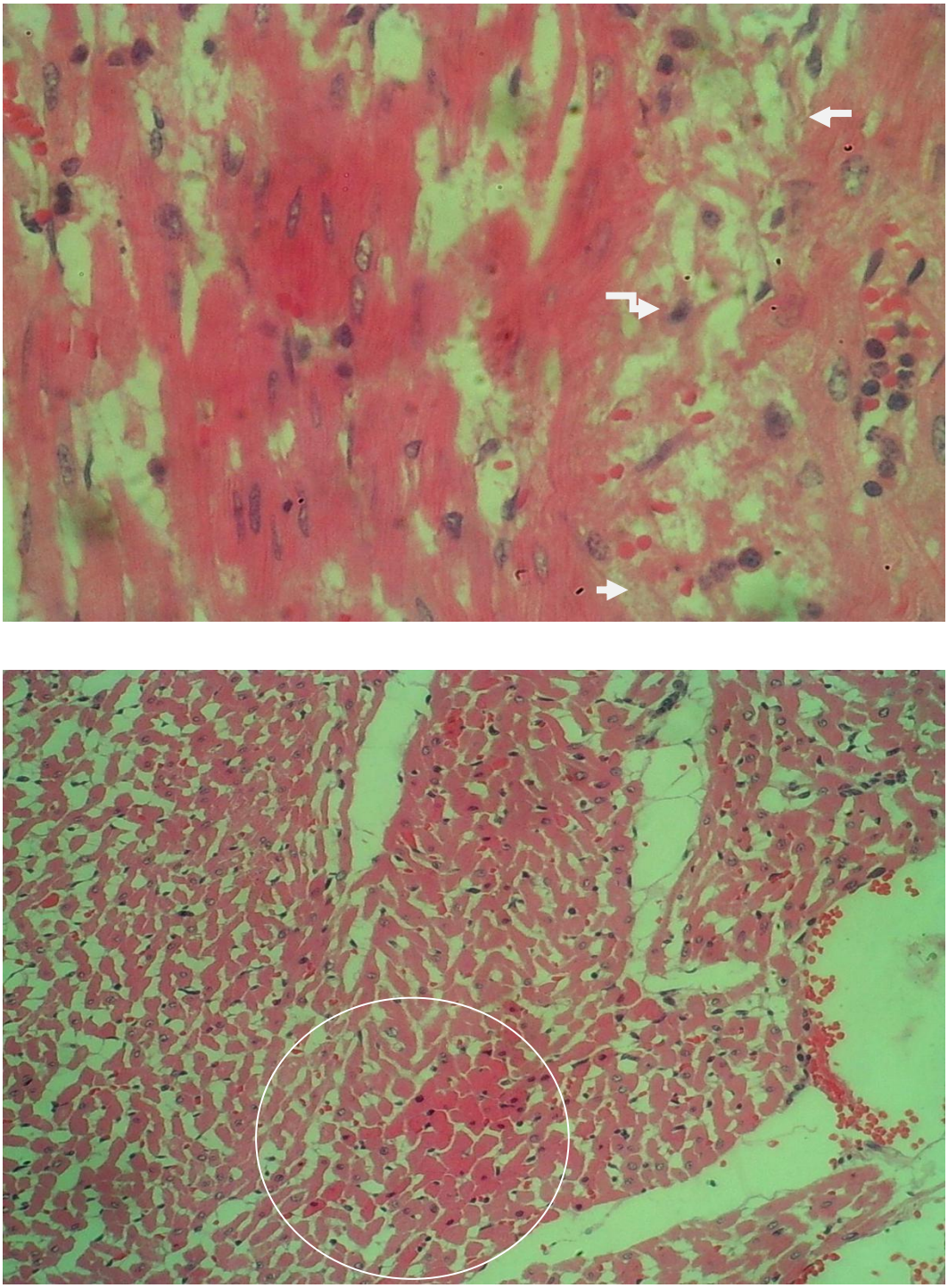
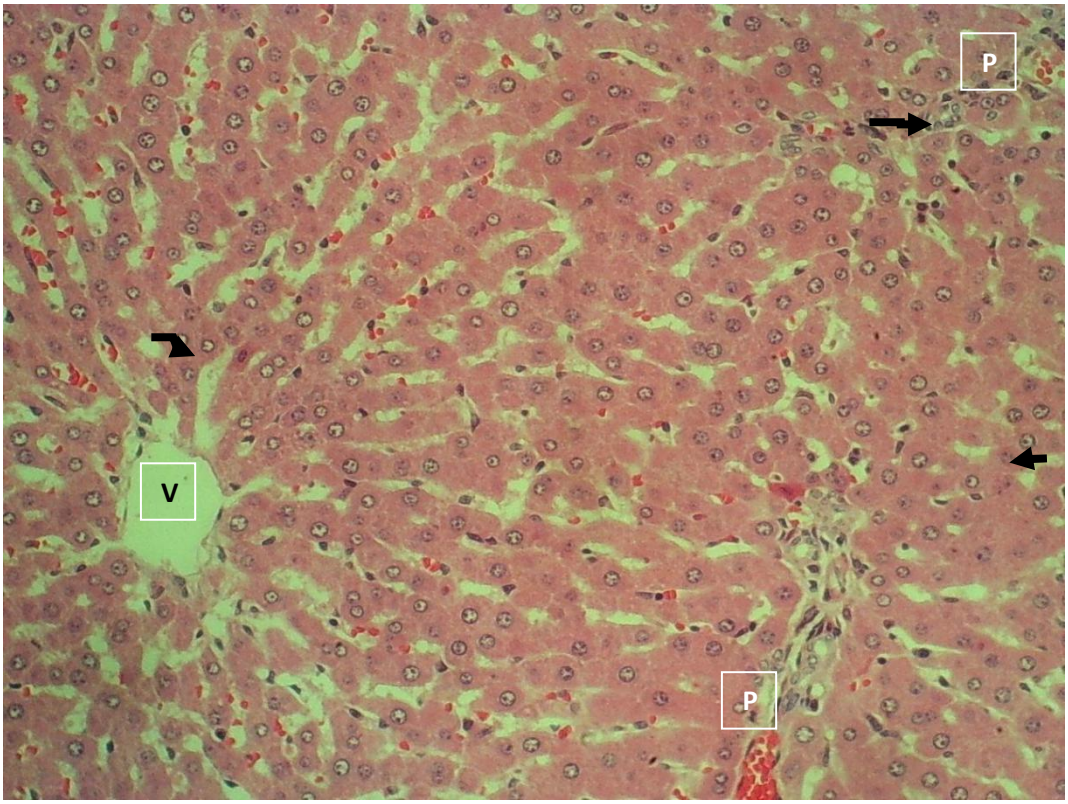


Figure 12: Sections of the heart collected from rats in Positive control group showed moderate multifocal areas of myocardial necrosis. The affected areas showed marked fragmentation of the muscle fibres (arrow) with loss of striations and nuclear karyolysis as well as typical Zenker's necrosis (circled). H&E x160;x400.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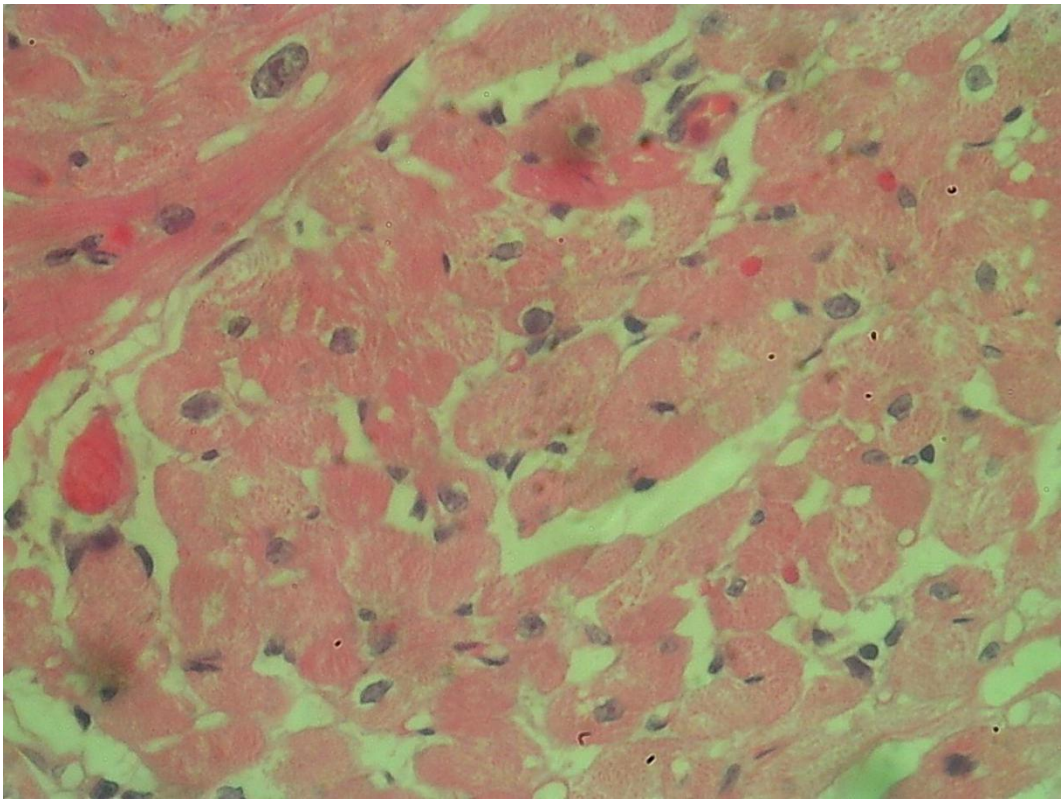

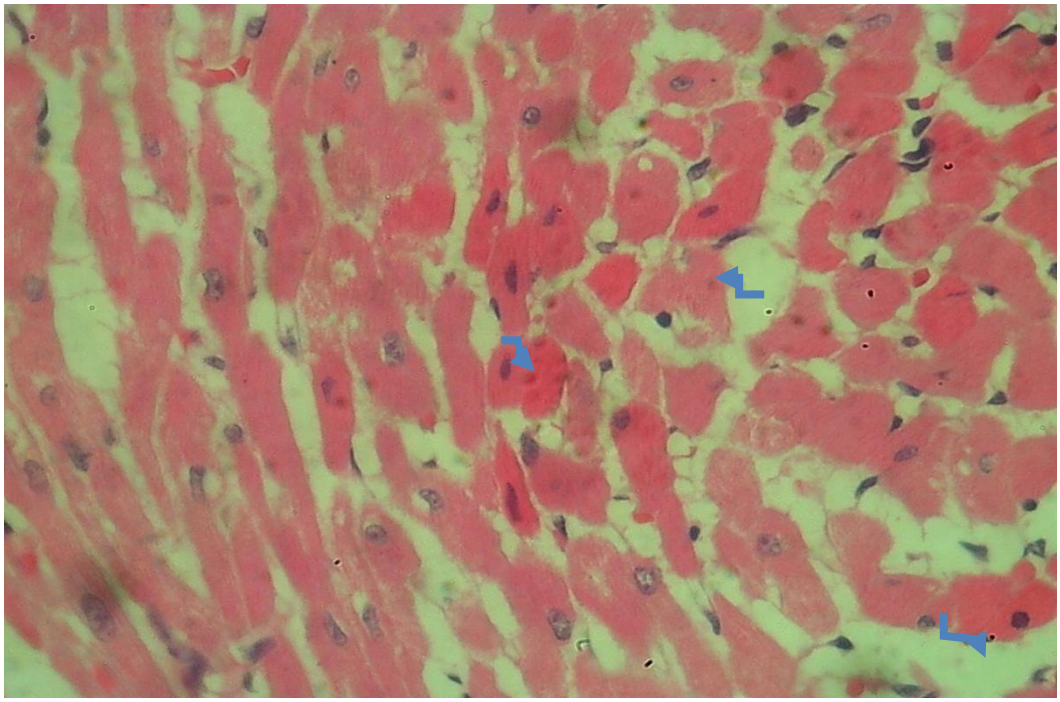

Figure 13: The section of the heart collected from rats in the group administered 200mg/kg extract showed, moderately widespread areas of myocardio-cellular degeneration. The affected areas showed relatively swollen muscle cells with pale cytoplasm, variably sized clear cytoplasmic vacuoles and loss of cross striations (black arrow). Admixed within these areas are muscle cells showing typical myocardial necrosis known as Zenker's necrosis (blue arrow). H&E x160.

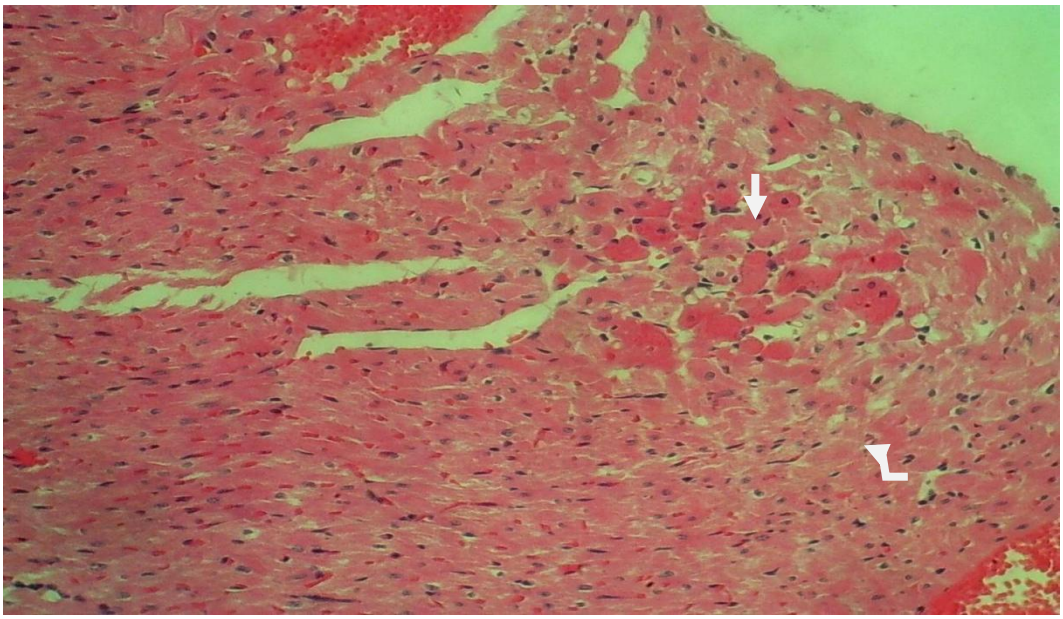

Figure 14: Sections of the heart collected from rats in the group administered 400mg/kg extract showed a few multifocal areas of myocardio-cellular degeneration (arrow). H&Ex160.

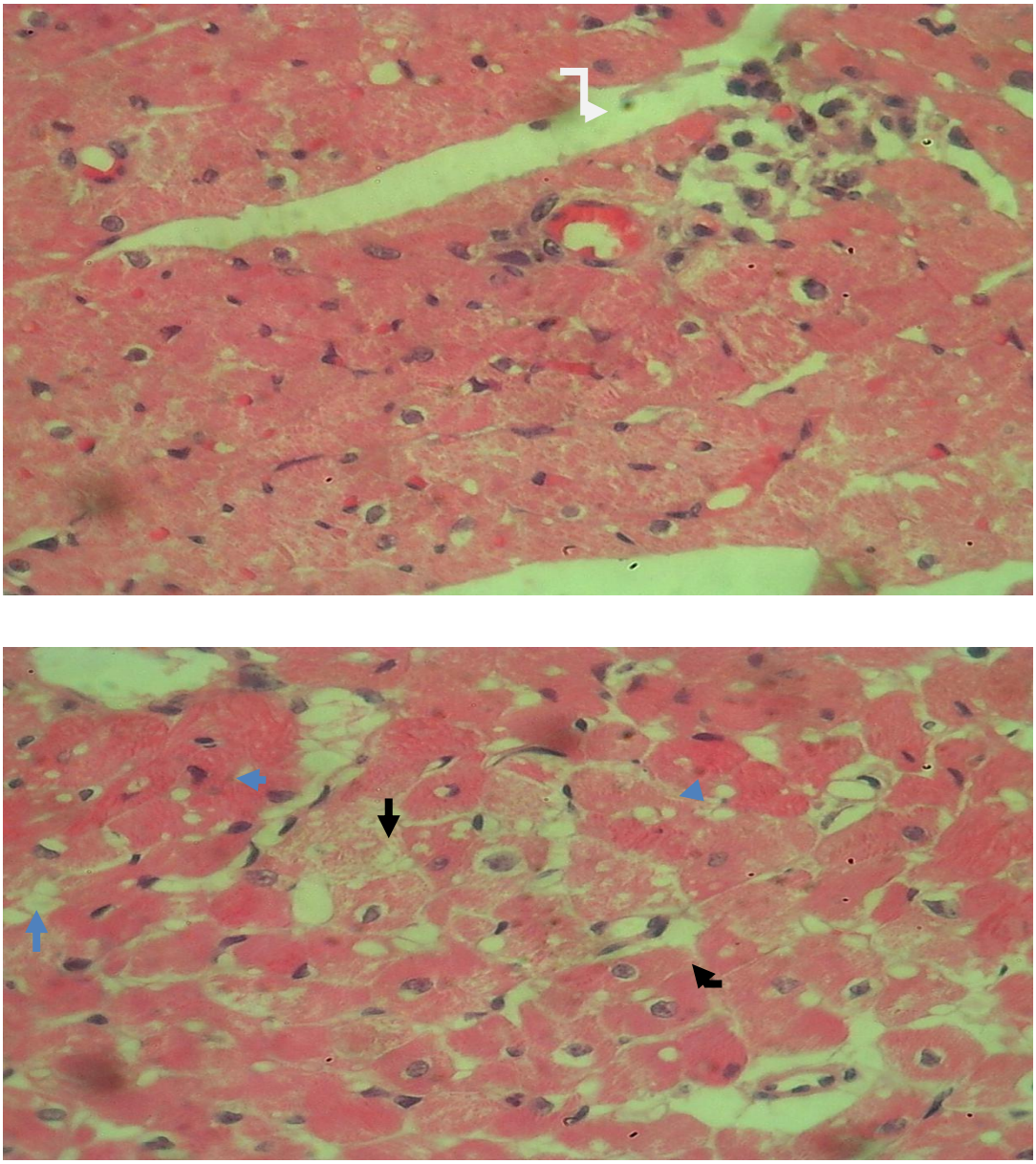

Figure 15: Section of the heart collected from rats in the group administered 600mg/kg of the extract showed multiple histomorphological alterations consistent with cardiotoxicity. Mild multifocal myocardial necrosis with infiltration of mononuclear leucocytes was observed (white arrow). Also, multifocal areas of myocardio-cellular degeneration were observed. The affected muscle cells appear swollen with pale cytoplasm, variably sized clear cytoplasmic vacuoles and loss of cross striations (black arrow). Admixed within these areas are muscle cells showing typical myocardial necrosis known as Zenker's necrosis (blue arrow). The affected areas showed dissociation, fragmentation and clumping of the myocardial cells, loss of cross striations, cytoplasmic eosinophilia and nuclear pyknosis. H&E x160.

DISCUSSION

In this study, the effect of methanol leaf extract of *Jatropha tanjorensis* on cardiac function biomakers, endogenous antioxidant activity and architecture of the heart in isoprenaline induced myocardial infarction in rats were evaluated.

The *in vitro* phytochemical analysis revealed the presence of important bioactive compounds such alkaloid, tannin, flavonoid, saponin, phenolics terpenoid and steroid. The acute toxicity of methanol leaf extract of *Jatropha tanjorensis* in mice recorded no mortality even at a high dose of 5000mg/kg of the extract, thus LD_{50} of the leaf could not be determined.

Cardiovascular diseases are globally known as the major cause of morbidity and mortality in the modern era (Syeda and Vasudeva, 2018). Myocardial infarction is a situation whereby there is significant decrease or block in the blood (Oxygen) supply to the part of the heart, leading to degeneration of a portion of the myocardium which triggers a caseade of cellular inflammatory and biochemical events, leading to the irreversible death (necrosis) of the muscle cells (Syeda and Vasudeua, 2018). Isoprenaline is a synthetic catecholamine and a non-selective beta adrenergic agonist with low affinity for alpha adrenergic receptor which produces infarct like necrosis of myocardium in high dose (Kharadi *et al*., 2016). Many authors have shown that isoprenaline has the ability to cause myocardial infarction at high dose (Radihika *et al*., 2013; Neha and Lubna, 2014; Abi *et al*., 2014, Kaksha *et al*., 2015, Mtopi *et al*., 2019). Though the mechanism by which isoprenaline induces myocardial infarction is not proven yet. It is hypothesise that intracellular calcium overload, alteration of myocardial cell membrane permeability due to lipid peroxidation (Abi *et al*., 2019), hypoxemia due to increase cardiac work and oxygen demand, free oxygen radical generation by auto oxidation of catecholamines, mitochondrial oxidative phosphorylation interruption by free fatty acid and changes in electrolyte content could be the possible mechanism (Kaksha *et al*., 2015).

Cardiac function biomarkers are proteins/enzymes that are used as essential tools in cardiology for primary and secondary prevention, diagnosis and management of acute myocardial infarction and other heart related issues (Johannes *et al*., 2015).

The result of the troponin I and CK-MB activity were represented in a chart in figure 1-4. Troponins are regulatory protein found in skeletal and cardiac muscle that is integral to muscle contraction. Cardiac troponin 1 control the calcium mediated interaction between

actin and myosin (Rachel, 2016). Troponin I is commonly use in the identification of cardiac muscle damage (Amsterderm *et al*., 2014; Rachel, 2016). Creatine kinase (CK) is an enzyme that catalyzes the reversible transformation of creatine and ATP to creatine phosphate and ADP (McLeish and Kenyon, 2005). There are three types of CK called isoenzymes: CKMM, found in skeletal muscle and heart; CK-MB, found in the heart and CK-BB, found mostly in the brain (Rosalki *et al*., 2004). CK-MB rises in the serum at 4-9 hours after the onset of chest pain or cardiac cell damage (Sabesan and Narasimhan, 2015).

From this study, it was observed that the negative control group proved myocardial necrosis/cardiotoxity with its significant ($P<0.05$) increase in troponin 1 and CK-MB activity. This is in line with the research of several authors (Vibha *et al.*, 2011; Abi *et al*., 2014; Mtopip *et al*., 2019). The marked increase in the troponin I and CK-MB level of the negative control shows that there was damage in the myocardial tissue caused by isoprenaline.

Sabeenaz *et al*. (2004) reported increase in troponin 1 level of metabolically damaged cardiac cells, there is also release of cardiac marker enzymes into the extracellular fluid in myocardial necrosis of the heart (Sabeenz *et al*., 2004). Nicholas and Alan (2015) reported that increased cardiomyocyte stretch and plasma membrane permeability can lead to the release of troponin I from the cytosolic pool. Also increase cell wall stress can lead to cardiomyocyte apoptosis and breakdown of contractile apparatus thus releasing troponin I (Hessei *et al*., 2008). Disruption of the cell membrane due to hypoxia or other injury releases CK-MB from the cellular cytosol into the systemic circulation.

This study suggested that *Jatropha tanjorensis* could be cardiotoxic at high dose based on the significant increase in troponin I and CK-MB activity of group administered 600mg/kg of the extract. The group administered 400mg/kg of the extract showed non-significant ($p<0.05$) increase in the troponin I and CK-MB level when compared with the positive control. The extract showed moderate ability to prevent elevation of troponin I and CK-MB activity at its 400mg/kg dose.

Lactate dehydrogenase (LDH) is found in almost every tissue especially in skeletal muscle, heart, liver, kidney, brain, lungs and red blood cells. Serum LDH activity is an indicator of cell damage and increase in LDH level occur in association with a wide variety of diseases (Brian *et al*., 2013). There was non-significant ($p>0.05$) increase in the LDH activity of the

extract treated groups when compared to the positive control. The negative control group significantly increases the LDH activity.

High sensitive C reactive protein (hsCRP) has been reported as a marker of systemic inflammation. It is elevated in most myocardial infarction patients (Juan *et al*., 2019). hsCRP is associated with subsequent risk of major adverse cardiovascular events and death (Juan *et al*., 2019). The contribution of inflammation to the pathophysiological feature of atherosclerosis is well established (Gomez *et al*., 2018; Tunon *et al*., 2018), as well as the use of hsCRP for predicting the risk of vascular event in cardiovascular prevention (Ridker, 2018). The significant ($p<0.05$) increase in the hsCRP level of the negative control group in this study is an evidence that there is inflammation associated myocardial infarction as reported by Juan *et al*. (2019).

The extract at 400mg/kg dose significantly ($p<0.05$) reduced hsCRP level compared to the positive control. This increase in the positive control implies that the carvedilol at 2mg/kg dose could not prevent inflammation caused by administration of Isoprenaline. Shigeru *et al*. (2019) reported that carvedilol has less anti-inflammatory property and the report validate the finding from this study.

So many health challenges are due to over secretion of reactive species in the body system. Reactive species are produced in the cell during normal cellular metabolism and can chemically react with cellular biomolecules such as nucleic acids, proteins, and lipids, thereby causing their oxidative modifications leading to alterations in their compositions and potential damage to their cellular activities. Fortunately, cells have evolved several antioxidant defense mechanisms (as metabolites, vitamins, and enzymes) to neutralize or mitigate the harmful effect of reactive species and/or their byproducts. Any perturbation in the balance in the level of antioxidants and the reactive species results in a physiological condition called “oxidative stress (Ankita *et al*., 2019). Oxidative stress has been identified as root cause of the development and progression of several diseases (Deepak *et al*., 2015). Free radicals are also linked to the manifestation of many degenerative diseases. The formation of excessive reactive oxygen species (ROS) can induce oxidative stress, leading to cell damage that can result to cell death (Borut *et al*., 2013). Injury of myocardium due to ischemia includes cardiac contractile dysfunction, arrhythmias as well as irreversible myocyte damage. These changes are considered to be the consequence of imbalance between the formation of oxidant and availability of endogenous antioxidants in the system.

Lipid peroxidation is useful for the determination of oxidative stress because the hydroxyl radical is the reactive form of ROS and can initiate lipid peroxidation by attacking polyunsaturated fatty acid (Le, 2014). malondialdehyde (MDA) is one of the most commonly used biomarker for lipid peroxidation. MDA exhibit high reacting and ability to form adduct with many biological molecules (Fumiaki *et al*., 2019).

The MDA level of the negative control in this study significantly ($p<0.05$) increased when compared with the treated groups. This suggested manifestation of oxidative stress induced by isoprenaline. The non-significant ($P>0.05$) increase in MDA level of the extract treated groups when compared with the positive control is an indication that methanol leaf extract of *Jatropha tanjorensis* could prevent isoprenaline induced oxidative stress.

It is well known that the body encloses a complex of endogenous enzymatic antioxidant such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and many others (Ighodara and Akinloye, 2018). Glutathione peroxidase is an important intracellular enzyme that is responsible for the breakdown of hydrogen peroxides (H_2O_2) to water; and lipid peroxides to their corresponding alcohols, mainly in the mitochondria and in some cases, in the cytosol (Goth *et al.*, 2004). Thus, GPx plays a more crucial role of inhibiting lipid peroxidation process, protecting cells from oxidative stress (Gill and Tuteja, 2010).

There was significant decrease in catalase activity of the negative control and the group administered 600mg/kg of the extract. This decrease in catalase suggested that the extract at 600mg/kg dose may not be effective in suppressing hydroxy radical (H_2O_2) mediated cell damage caused by isoprenaline. A catalase is one of the crucial antioxidant enzymes that mitigate oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen. Deficiency or malfunction of catalase is postulated to be related to the pathogenesis of many age-associated degenerative diseases (Ankita *et al*., 2019). This suggested that the extract at high dose could promote hydroxyl radical mediated diseases. The study period may have limited any alterated in the SOD, GPx and GSH activity of the 600mg/kg extract treated group.

The 200mg/kg and 400mg/kg dose of the extract revealed non alteration in the antioxidant activity when compared with the positive. This suggested that the extract at said dose could prevent hydroxyl radical and superoxide radicals mediated cell damage. Glutathione plays a crucial role in the antioxidant defense system; removing free-radical species, such as

hydrogen peroxide and superoxide radicals as well as maintaining membrane protein thiols (Traber and Stevens, 2011).

The histopathological study in this research showed that all except the normal control have different degrees of cardiac alteration. Treatment of the experimental animals with isoprenaline caused widespread myocardial necrosis, multifocal area of myocardial cell degeneration, swollen muscle cells with pale yellow cytoplasm, loss of cross striation and fragmentation of the myocardial cells.

The section of the heart collected from the negative control and group administered 600mg/kg of the extract showed more cardiac cell damage compared to other groups which could be attributed to the depletion of catalase and increase MDA that was observed.

CONCLUSION

The methanol extract of *Jatropha tanjorensis* leaves at 400mg/kg dose prevented biochemical changes induced by Isoprenaline though there was mild alteration in the myocardial cells as observed in the histopathology of the heart. The extract at 600mg/kg dose could not prevent cardiac cell damage induced by Isoprenaline as observed in the histopathological study.

The significant ($p<0.05$) decrease in the cardiac biomarker (CK-MB, Troponin I, LDH, hsCRP) as well as significant increase in catalase, SOD, GPx, GSH with decrease in MDA suggested that the extract to some extent possess mild cardioprotective potency at certain dose range but could not serve as a potential agent for the prevention of cardiotoxicity. The result of this study revealed the need for proper dosing of crude drug (plant extract) as its high/over dose could be detrimental to health.

Conflict of interest

The authors declare that no conflict of interest exists with respect to this work.

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