

Hepatoprotective and Antidyslipidemia Effect of *Jatropha Tanjorensis* Leaf Extract in Isoprenaline Induced Myocardial Infarction in Albino Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: 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 study assessed the protective outcome of methanol extract of *Jatropha tanjorensis* leaf on liver function biomarkers and lipid profile condition in the isoprenaline induced myocardial infarction in albino rats.

Methods: Thirty six (36) rats were divided at random into six (6) groups of six rats per group. Group 1 was normal control, group 2 was negative control (85mg/kg of isoprenaline only), group 3 positive control (pretreated with 2mg/kg carvedilol for 28 days), group 4 through 6 were pretreated with 200mg/kg, 400mg/kg and 600mg/kg of methanol leaves extract of *Jatropha tanjorensis* respectively for 28 days.

Results: The extract at 200mg/kg and 400mg/kg significantly ($p < 0.05$) decrease the AST and ALT activities, TAG and Cholesterol concentrations compared to negative control. The extract treated groups also showed significant ($p < 0.05$) increase in the HDL concentration. The histopathological study showed marked atrophy of hepatic cord in the 600mg/kg of the extract treated group. This pointed that the extract at 600mg/kg dose could not possess hepatoprotective potency. The 400mg/kg dose extract ameliorated damage cells.

Conclusion: This study revealed that 400mg/kg of the extract could serve as an agent for the prevention of hepatic cell injury and dyslipidemia.

Keywords: Hepatotoxicity, antidyslipidemia, Myocardial infarction. Protective, *Jatropha tanjorensis*

INTRODUCTION

The Prevalence of Myocardial infarction as the prim cause of death worldwide is a public health challenge. Myocardial infarction is a disorder in which there is cardiac muscle cell necrosis due to substantial and persistent ischaemia. It is normally, but not always an acute manifestation of atherosclerosis-related coronary heart diseases, which implies obstructing mechanisms (Mendis *et al.*, 2011).

Ischemic heart disease is a major cause of cardiovascular disease (Shen-Jie *et al.*, 2015) and there

is a correlation between high level of plasma total cholesterol, triacylglycerol with high risk of atherosclerosis and cardiovascular disease owing to hepatic insufficiency (Ekaidem *et al.*, 2007).

The vascular organ that play vital role in metabolism, detoxification and excretion is known as the liver. This vital organ called the liver metabolizes substance via various biochemical pathway including hydration, condensation, oxidation, reduction, hydrolysis or conjugation, alteration in any of the aforementioned process may result to liver cell injury (Sriuasfava and Sriuasfava, 2018).

Liver dysfunction is associated with cardiac disease such as myocardial infarction (Lightsey and Rockey, 2017). Blockage of blood flow and congestion can manifest to liver damage (Biegus et al., 2012), and the damaging effect of the myocardial infarction on the liver are multifactorial including decrease in blood flow to the liver, reduced arterial saturation and increased hepatic vein pressure (Ambrosy et al., 2012). Liver disease possibly may be inflammatory, non-inflammatory and degenerative.

The usefulness of medicinal plants in combating many health challenges and its utility in pharmaceutical industries have been reported; among these plants is *Jatropha tanjorensis*. *Jatropha tanjorensis* known as Chaya leaf and generally known as 'Hospital too far' in Nigeria, is a shrub from the family Euphorbiaceae. Different parts of *Jatropha* plants are utilized in some ways and in several countries.

METHODOLOGY

Collection of Plant leaves

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

Pulverizer ((5126 TP) was used to pulverized the sample into fine powder and preserved in cellophane bags until when used.

Reagents/Chemicals used

All the laboratory 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

A method described by Unegbu et al (2017) was used in the preparation of the extract.

Experimental animals

Thirty-six (36) male albino rats aged 10-12 weeks with 80-120g in weight and 18 mice weighing 16-22g were acquired from the animal farm of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in standard transparent cages with wheat husk bedding, renewed every 24h. The rats were kept under humid tropical condition and acclimatized for two

Jatropha tanjorensis leaves has been reported to possess numerous medicinal properties such as hepatoprotective (Ezeonu et al., 2017), antidiabetes (Chinenye et al., 2019), anticancer (Purshothaman et al., 2014), antianaemic (MacDonald et al., 2014), antiulcer (Arumugam et al., 2016), hypolipidemic (Oyewole and Akingbala, 2011), antioxidant (Omoriegie and Osagie, 2012), antibacterial (Danianyan et al., 2018), among others.

Been that very few publications have been reported about the outcome of *Jatropha tanjorensis* on the liver and lipid profile of isoprenaline induced myocardial infarction, the present study evaluated whether *Jatropha tanjorensis* leaf extract has hepatoprotective and antidyslipidemic effect against ischemic myocardial infarct model in albino rats.

weeks to laboratory conditions. The guidelines given by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and NRC were observed and the protocol was approved by institution ethical committee for the use of laboratory animals with Ethical number BCM/EC/02/072. .

Acute Toxicity (LD₅₀)

The acute toxicity of the leaf was determined using Lorke's method (Lorke, 1983).

Experimental Design

Total 36 albino rats were allocated randomly into 6 groups (6 animals per group). Hepatic injury 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 24h. Distribution of study groups were as follow:

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

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

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

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

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

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

After the treatment period, cervical dislocation method was used to sacrifice the rats and blood samples collected through cardiac puncture using 2ml syringes. 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 liver was further harvested for histopathological studies.

Estimation of AST and ALT level in the serum

Serum ALT and AST activity were estimated by the method of Reitman and Frankel (1957)

RESULTS AND DISCUSSION

Phytochemicals screening

The *in vitro* phytochemical analysis revealed the presence of important bioactive compounds such alkaloid, tannin, flavonoid, saponin, phenolics terpenoid and steroid.

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

Phytochemicals	Inference
Phenols	+
Flavonoid	++
Saponin	+

Lipid profile

Serum total cholesterol concentration, Serum HDL-cholesterol level and Triacylglycerol were ascertained using the method of Albers *et al* (1978) as contained in QCA commercial kits.

Histopathological examination

Tissue preparation

The liver was preserved in 10% phosphate buffered formalin and a method described by Sarowoot and Chuchard (2013) with slight modification was used for histopathological studies

Statistical analysis

The data obtained were statistical analysis with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The data were presented as Mean+SEM. 95% confidence level of probability ($P < 0.05$) was considered.

Alkaloid	++
Tannins	+
Terpenoid	+
Steroid	+

+ = present, ++ highly present.

Acute toxicity studies

Result of acute Toxicity Study suggested that the *Jatropha tanjorensis* leaf extract does not have any acute toxicity since no mortality was recorded at the highest dose of 5000mg/kg.

Table 2: Result of acute Toxicity Study of methanol extract of *Jatropha tanjorensis* leave

Groups	Concentration (mg/kg)	Mortality/signs of toxicity
Phase 1		
1	10	Nil
2	100	Nil
3	1000	Nil
Phase 2		
1	1600	Nil
2	2900	Nil
3	5000	Nil

No mortality was recorded in the acute toxicity study as shown (table 2)

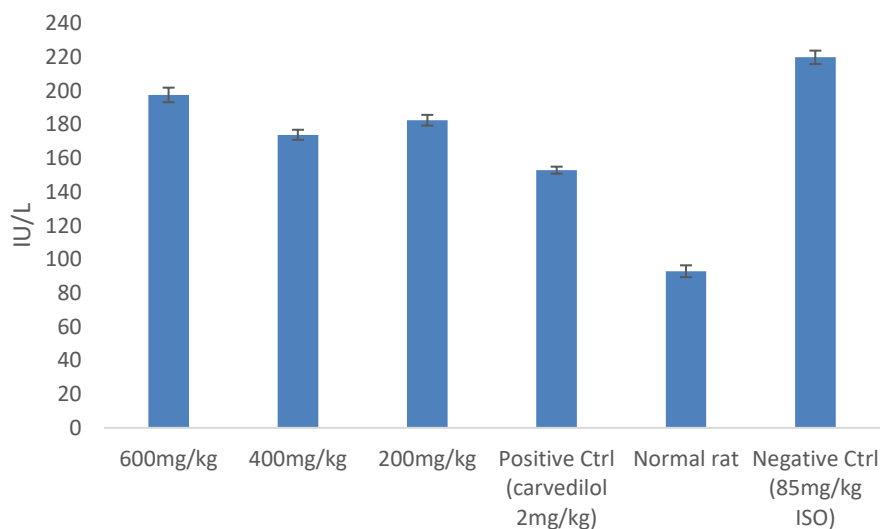


Figure 1: AST Activity of Isoprenaline induced liver injury followed myocardial infarction in rats

The extract treated groups significantly ($p < 0.05$) decrease AST activity when compared with the negative control. The AST activity in the group treated with 400mg/kg of the extract revealed non-significant

($p < 0.05$) increase when compared with the positive control (figure 1).

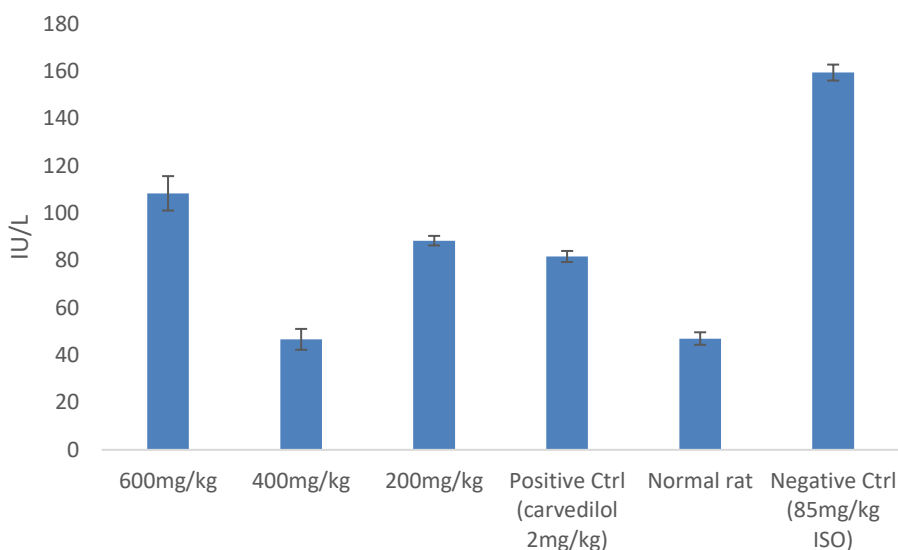


Figure 2: ALT Activity of Isoprenaline induced liver injury followed myocardial infarction in rats

The result of the ALT activity shows significant ($p < 0.05$) decrease in the group administered with extract compared with the group used as negative control (85mg/kg ISO only) group. There was significant ($p < 0.05$) increase in the group treated with

600mg/kg of the extract when compared with positive control and there was non-significant ($p > 0.05$) difference in the group treated with 400mg/kg extract and normal rats (figure 2).

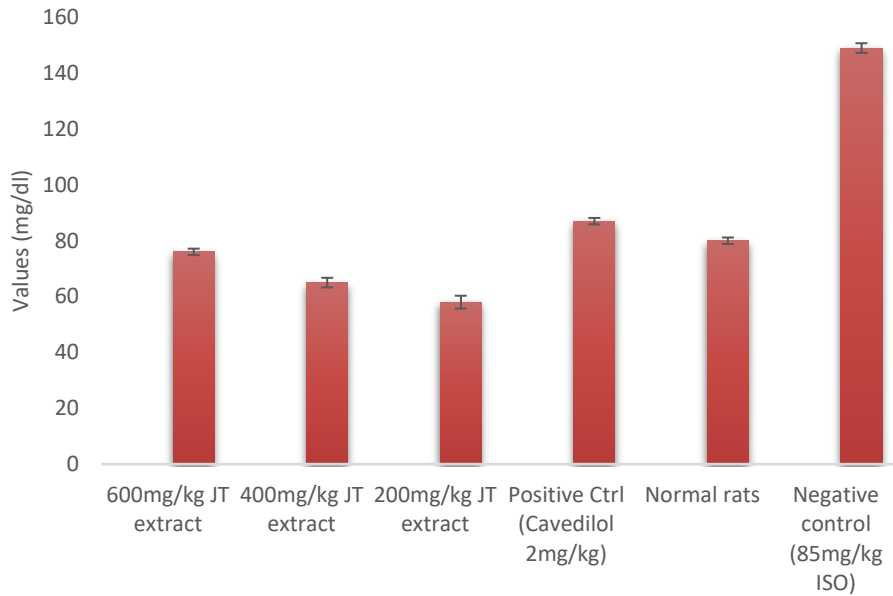


Figure 3: TAG level of Isoprenaline induced liver injury followed myocardial infarction in rats

There was significant ($p < 0.05$) decrease in the TAG concentration of the group administered with 400mg/kg and 200mg/kg of the extract when compared with all the control groups. There was non-

significant ($p > 0.05$) increase in the groups administered 600mg/kg of the extract when compared with 2mg/kg of carvedilol (positive control) and normal control group (figure 3).

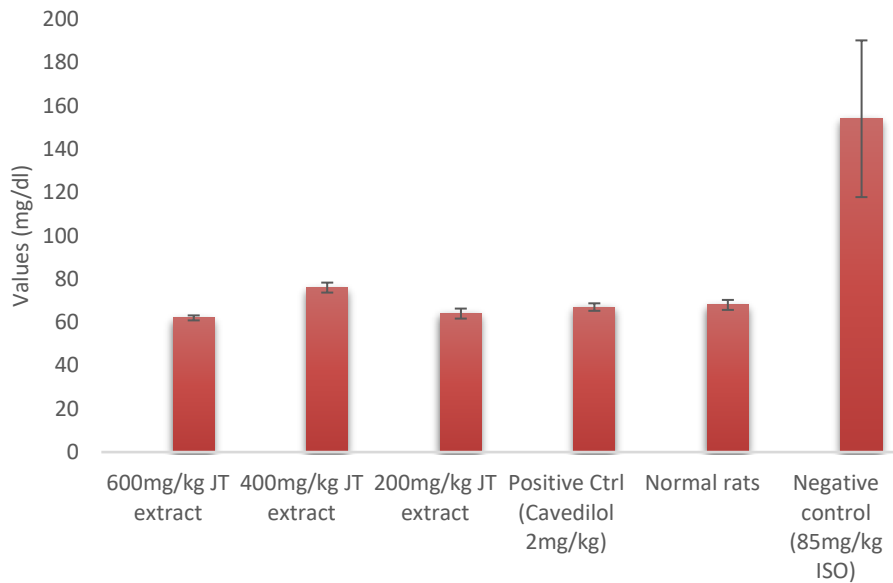


Figure 4: Total cholesterol level of Isoprenaline induced liver injury followed myocardial infarction in rats.

The result of the total Cholesterol concentration shows non-significant ($p > 0.05$) increase in the group administered 200mg/kg, 400mg/kg and 600mg/kg of the extract when compared with the positive control.

The extract treated groups revealed significant ($p < 0.05$) decrease when compared with the negative control (85mg/kg ISO only) group (figure 4).

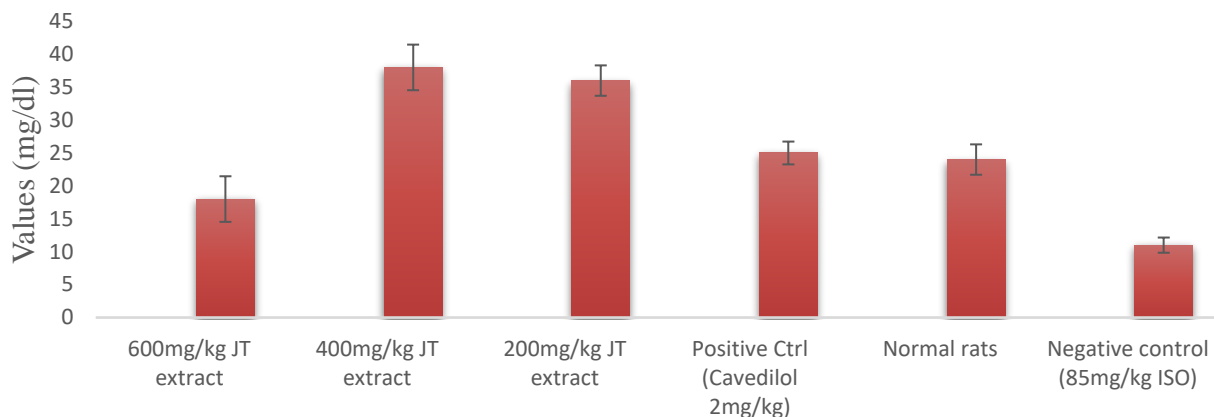


Figure 5: HDL level of Isoprenaline induced liver injury followed myocardial infarction in rats

The HDL level in the groups administered 200mg/kg and 400mg/kg of the extract was shown significant ($p < 0.05$) increase when compared with the control groups. The groups administered 600mg/kg of the extract revealed significant ($p < 0.05$) decrease when compared with the positive control and normal control.

The histopathological study showed that the group administered 600mg/kg of the extract (plate 6) and the negative control (plate 2) showed widespread atrophy of the hepatic cords with consequent accentuation of the hepatic sinusoids which implies hepatic cell injury. The positive control (plate 3) as well as groups administered 200mg/kg and 400mg/kg of the extract (plate 4 and 5 respectively) showed normal hepatic histomorphology for the laboratory animals.

Histopathological study

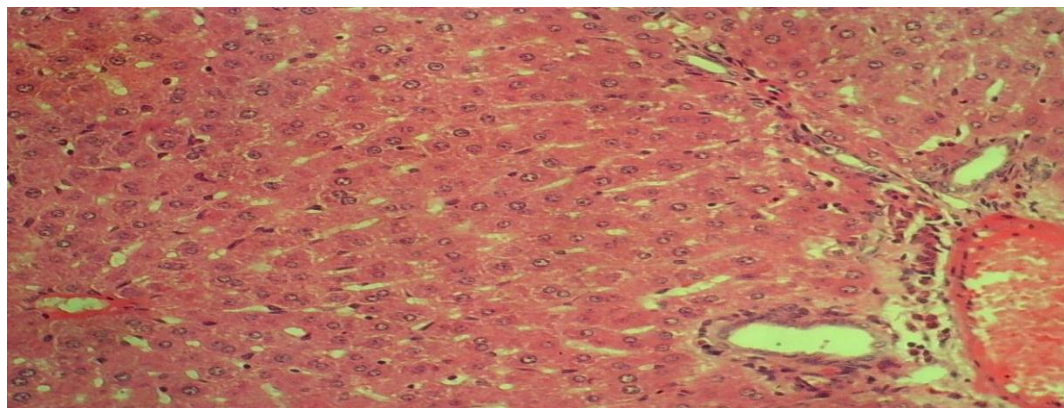


Plate 1: Sections of the liver collected from rats in Normal control group showed the normal hepatic histomorphology for laboratory rodents. Central vein (V); hepatic vein (HV); Bile duct (BD). H&E x16

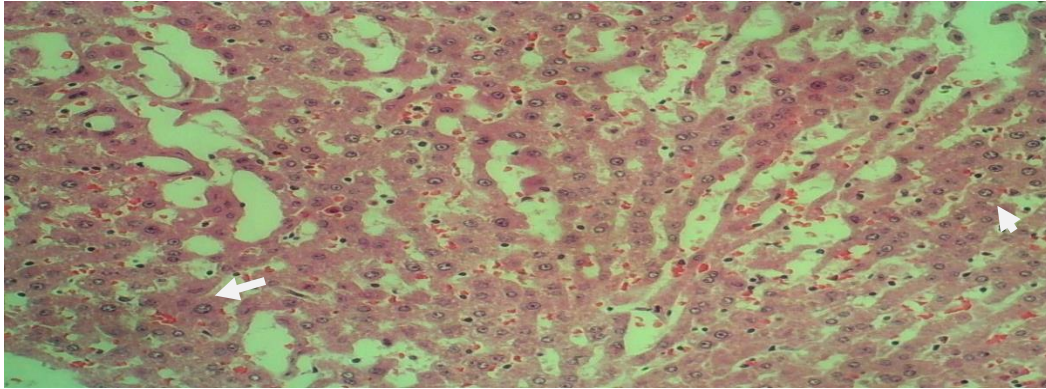


Plate 2: Sections of the liver collected from rats in Negative control group showed widespread atrophy of the hepatic cords with consequent accentuation of the hepatic sinusoids (arrow). H&Ex160

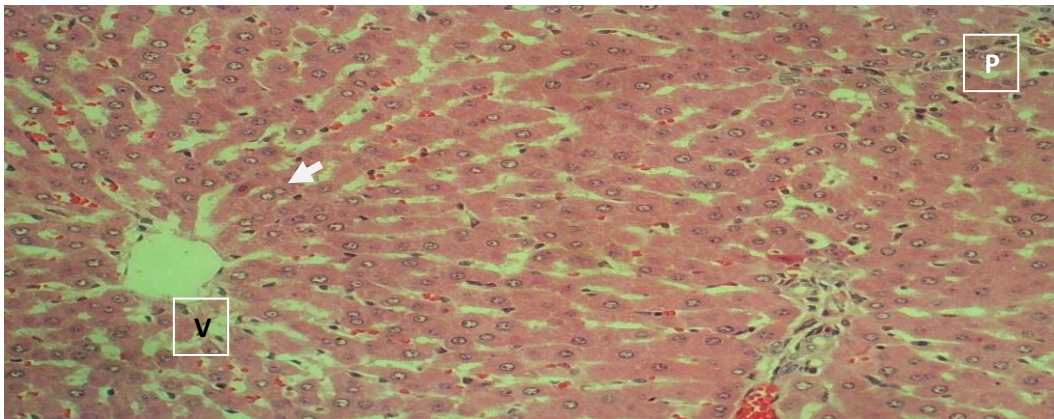


Plate 3: Sections of the liver collected from rats in Positive control group showed the normal histomorphology of the liver. Central vein (V); Portal area (P).H&E x160

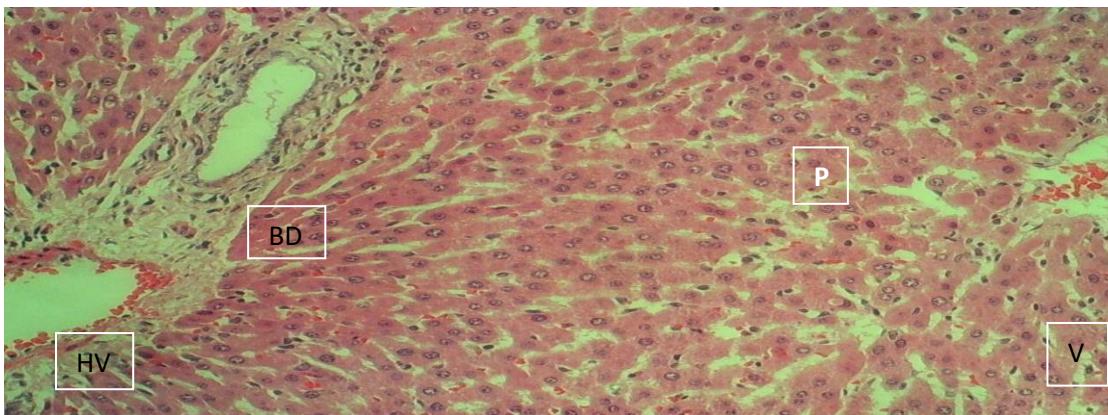


Plate 4: Sections of the liver collected from rats in the group administered 200mg/kg of the extract showed the normal hepatic histomorphology. Central vein (V). Components of the portal area (Hepatic vein –HV; Bile duct –BD). H&E x160.

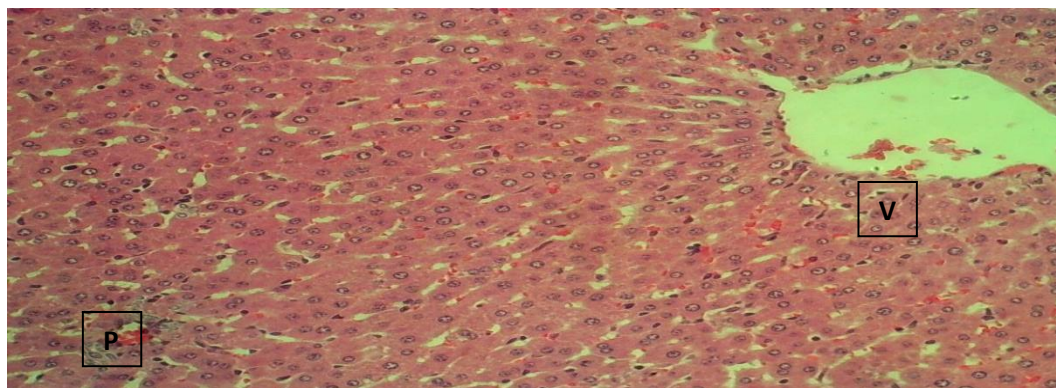


Plate 5: Sections of the liver collected from rats in the group administered 400mg/kg extract showed the normal histomorphology of the liver. Central vein (V); Portal area (P).H&E x160

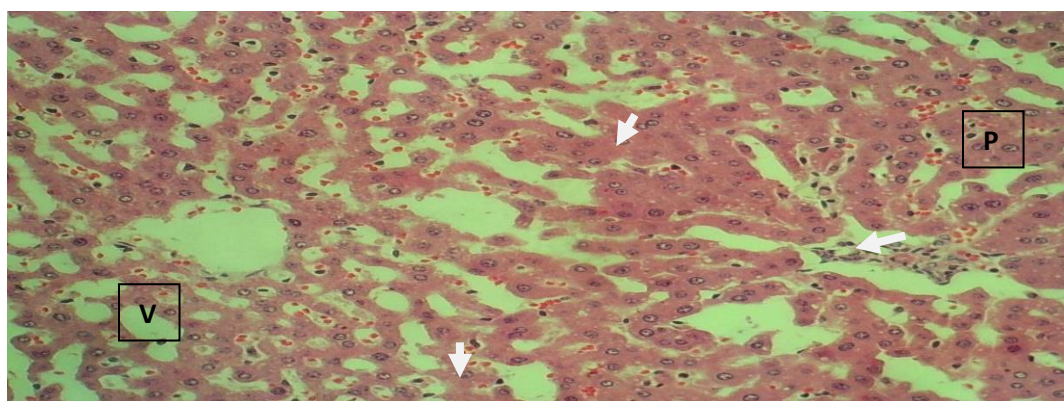


Plate 6: Sections of the liver collected from rats in the group administered 600mg/kg of the extract showed widespread atrophy of the hepatic cords with consequent accentuation of the hepatic sinusoids (arrow). Central vein (V); Portal area (P). H&Ex160

DISCUSSION

The effect of methanol extract of *Jatropha tanjorensis* leaf on lipid profile, liver enzymes and architecture of liver in isoprenaline induced myocardial infarction in rats were evaluated.

The *Jatropha tanjorensis* possess flavonoid and tannin as part of the phytochemical constituents, this two bioactive compounds are well known for their hepatoprotective potency (Rehman *et al.*, 2015). Saponin, Alkaloids, Terpenoids and flavonoid are well known antioxidants and have been reported to be useful the management of oxidative stress mediated diseases (Jin *et al.*, 2011). 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 LD₅₀ of the leave could not be determined.

Cardiovascular diseases are the major cause of morbidity and mortality in the modern era. 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 could consequently triggers a cascade of cellular inflammatory and biochemical events, resulting in irreversible death (necrosis) of the muscle cells (Syeda and Vasudeva, 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). 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 (Dylan *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 (Theodor *et al.*, 2016).

There is a close relationship between Ischemic hepatitis and cardiovascular diseases, reports have shown a significant link between myocardial infarction and the risk of developing ischemic hepatitis; a condition that occurs as a result of decrease total hepatic blood flow secondary to low cardiac output, shock or cardiac arrest (Mason *et al.*, 2010). Increased serum liver enzymes are also associated with cardiovascular disease risk. ALT is mainly produced by the liver due to increased hepatic inflammation or injury whereas AST originate from liver and muscle cells and rises with myocardial cell injury or hepatic dysfunction (Kyung *et al.*, 2018). The present study revealed decrease in the AST and ALT value of the treated groups compared to the negative control. Increased AST and ALT value of the negative control compared to the normal control is a clear indication of cardiotoxicity/hepatotoxicity caused by isoprenaline. The reduced value in ALT and AST of the treated group is suggested as evidence of ameliorated potency of the methanol leave extract of *Jatropha tanjorensis* against myocardial and hepatic cell necrosis.

High density lipoprotein (HDL) is well known by its reverse cholesterol transport which aids the removal of excess cholesterol from the peripheral vessels and transported back to the liver for disposal (Kosmas *et al.*, 2018). Several prospective epidemiological survey have shown that there is inverse correlation between serum HDL-cholesterol concentration and risk of coronary heart disease (Barter *et al.*, 2007).

CONCLUSION

There is a close associated between dyslipidemia and myocardial infarction which could lead to alteration in liver function biomarkers. The methanol extract of *Jatropha tanjorensis* leaf at 400mg/kg and 200mg/kg doses showed ability to prevent alteration in liver

The increase in the HDL level in the treated groups in this study is an indication that the extract can prevent coronary heart disease. The increase in the total cholesterol and triacylglycerol (TAG) level of the negative control compared to the normal control and treated group is an evidence of dyslipidemia caused by isoprenaline. The decrease in the cholesterol and TAG level of the treated group suggested preventive efficacy of the extract against coronary heart disease and heart attack. Sabeena *et al* (2016) reported that isoprenaline has the ability to cause severe hyperlipidemia. This report confirmed the result observed in the negative control.

The histopathological study showed normal histomorphology of the liver in all groups except the negative control and group administered 600mg/kg of the extract. The liver collected from 600mg/kg group and negative control showed widespread atrophy of the hepatic cord with consequent accentuation of the hepatic sinusoid compared to other groups, this signifies hepatotoxicity. Oyewole *et al* (2012) reported that *Jatropha tanjorensis* leaf extract could disrupt protein metabolism function of the liver and also interfered negatively with the filtration capacity of the kidney which might result in renal and hepatic dysfunction. The alteration in liver morphology as seen in 600mg/kg of the extract is in line with the report and this proved that continuous intake of the extract at high dose could lead to alteration in functionality of vital organs. The 400mg/kg and 200mg/kg of the extract group showed ability to prevent hepatotoxicity and dyslipidemia.

function biomarkers and dyslipidemia in rats as well as maintained the architectural integrity of the rats' liver whereas 600mg/kg of the extract showed signs of hepatotoxicity. The findings encourage proper dosing of crude drug (plant extract) before use.

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