Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Cardioprotective Effect of Hydroalcoholic Leaf Extract of *Jatropha mollissima* on Isoproterenol-induced Myocardial Infarction in Rats

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Submitted: 10-Jan-2021 Revised: 27-Apr-2021 Accepted: 13-May-2021 Published: 15-Sep-2021

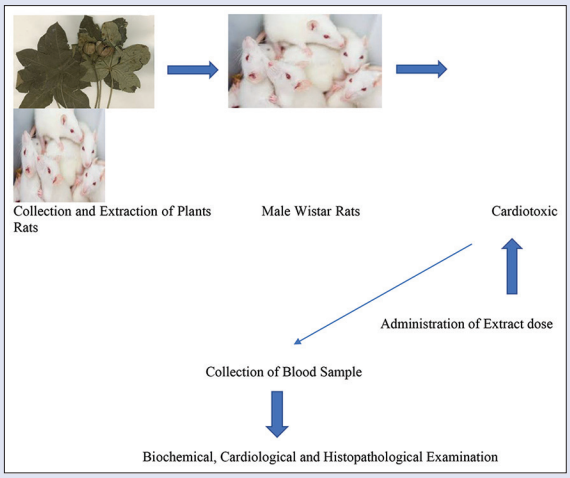
ABSTRACT

We aim to explore various effects of doses of *Jatropha mollissima* against isoproterenol-induced myocardial infarction in the rat. Rats were divided into 6 groups (10 rats in each). Group-1 was normal to control, and Group-2 was considered intoxicated isoproterenol (ISP) (100 mg/kg, s. c.) Group-3 was treated with standard drug carvedilol, while Group-4, 5, and 6 were treatment groups and treated with *J. mollissima* extract at the doses of 400, 600, and 800 mg/kg, respectively. Preliminary phytochemistry, histopathological variation in the myocardium, antioxidant potential, and cardiac biomarkers (serum glutamic oxaloacetic transaminase, triglyceride, 2,4,6-trinitrotoluene, serum glutamic pyruvic transaminase, lactate dehydrogenase, and creatine kinase muscle–brain fraction), cardiac rate, electrocardiographing, and pressurization rate index were estimated. This study indicates that the extract proved to have cardioprotective potential and significantly reduced the cardiac biomarkers in a dose-dependent fashion because of flavonoid contents and antioxidant property. The histopathological analysis shows marked improvement in *J. mollissima* groups handled in comparison with ISP. The present evaluation suggests that *J. mollissima* has exceptional cardioprotective potential contrary to toxicity caused by isoproterenol. We recommend further studies at the molecular level to frame the exact mechanism of action.

Key words: Cardioprotective, isoproterenol, *Jatropha mollissima,* reactive oxygen species

SUMMARY

- We aim to explore various effects of doses of *Jatropha mollissima* against isoproterenol induced myocardial infarction in the r at. The present evaluation suggests that *J. mollissima* has exceptional cardioprotective potential contrary to toxicity caused by isoproterenol



Abbreviations used: *J. mollissima*: *Jatropha mollissima*; B. W: Body weight; CVS: Cardiovascular system; SD: Standard drug; H. W: Heat weight; JM: *Jatropha mollissima*; s. c.: Subcutaneous; ROS: Reactive oxygen species; N: Normal; GFR: Glomerular filtration rate; ISP: Isoproterenol; NC: Negative control; PC: Positive control; LPO: Lipid peroxidation; SPD: Superoxide dismutase; CAT: Catalase; GTR/GLR: Glutathione reductase; GA: Gallic acid.

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DOI: 10.4103/pm.pm_16_21

Access this article online
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INTRODUCTION

Among other cardiovascular diseases (CVD), myocardial infarction (MI) is still a major threat to human life and is regarded as a leading global cause of death today. As such, clinical cardiology has become a significant concern.[1] MI is a general occurrence of ischemic cardiac disease. Despite rapid treatments in coronary artery disease (CAD), MI is still the biggest reason for mortality in the industrialized world and seems to be a global

Cite this article as: Iqbal MO, Khan IA, Manzoor A, Arshad S, Sial AS, Dar E, *et al.* Cardioprotective effect of hydroalcoholic leaf extract of *Jatropha mollissima* on isoproterenol-induced myocardial infarction in rats. Phcog Mag 2021;17:S251-6.

pathologic concern (CAD).[2] It occurs through increased demand for ventricular mitochondrial and lowers the required energy and nutriment through the cardiovascular distribution to the myocardium, heading toward structural damage; it is among the most deadly embodiments of cardiovascular events.[3] MI is an important issue of public health, not just in Western societies but also in developing nations, significantly impacting mortality.[4] The World Health Organization states that MI is estimated to be the world's leading cause of death by 2020.[5]

Isoproterenol is a beta-agonist, which serves as an effective regulator for cardiac hypertrophy and oxidation, a crucial factor for studying the possible positive effects on cardiac function of various drugs.[3] Isoproterenol causes many heart cellulitis mechanisms, including increased oxygen intake, inadequate use of oxygen, elevated calcium excess load and concentration, modified microvascular metabolism, increased cyclic adenosine monophosphate myocardial levels, skewed electrolyte environment, distorted membrane intelligibility, endothelial malabsorption, and increased lipid peroxide levels.[6] In a myocardial necrotic rat model, pathophysiological and anatomical aberrations are comparable to those of human MI include the formation of strongly free cytolytic molecules through self-oxidation of monoamine neurotransmitters as one of the key mechanisms proposed to understand ISO-induced cardiotoxicity.[7] Catecholamine oxidation forms a protonated complex that results in superoxide radicals and hydrogen peroxide development, causing protein, lipid, and DNA damage and increased tissue size when used in the presence of iron.[8] The free radicals can attack inside the membranes, producing peroxyl radicals and polyunsaturated fatty acids. These extremists can then target neighboring triglycerides causing an oxidative damage chain reaction (LPO). The end products of lipid hydrogen peroxide damage and cause more chronic inflammation and organ.[9] The intake of fresh fruit, vegetables, or plants with a rich natural antioxidant has been linked to long-term CVD preclusion in recent decades. Consequently, there was significant research scope into biologically active medicinal plants, with the general view that organic products are superior to their synthetic analogs in terms of effectiveness and protection.[10]

The *Jatropha* L. family (Euphorbiaceae) has a place with the subspecies Crotonoideae and Jatropheae clan comprising over 300 species and widely dispersed the tropics, what's more, equatorial areas of Africa and the Americas. The name Jatropha is gotten from the Greek words "Jatros" (specialist) and "trophe" (food), which conceivably corresponded with the therapeutic qualities of plants from this family.[11] In Africa, Asia, and Latin America, the species Jatropha is found in conventional pharmaceutical products for treating different diseases and as ornamental plants and energy crops. A variety of recognized types of the genus Jatropha have been recorded for their medicinal uses, chemical constituents, and biological activities, such as *Jatropha curcas, Jatropha elliptica, Jatropha gossypifolia, and Jatropha mollissima.*[12] *J. mollissima* is widely used in folk medicine for different purposes, mainly as antivenom therapy[13-16] and anti-inflammatory[17] remedies, healing[14] veterinary vermifuge[13] also cures kidney diseases and loss of appetite.[14] Most of the studies found that antioxidant,[18] antimicrobial,[19] and anthelmintic,[20] nephroprotective,[21] antibacterial, and antibiotic-modifying actions[22] are present in this plant. At the same time, there are various studies on the medicinal benefits of the ethanolic extract of *J. mollissima*. The toxicological *in vivo* effect of the plant extract has not been identified. Therefore, in the albino rat model, it is considered essential to determine the cardioprotective effect of *J. mollissima*. This toxicity study would be an essential basis for further studies in herbal medicine development for this plant. The present research has endeavored to authenticate their conventional use using *Jm* leaves ethanol extract in the Wistar albino rats against isoproterenol-induced MI.

MATERIALS AND METHODS

Animals

Wistar male rats were taken and held in cages, weighing 170–200 g. The study was carried out at the Pharmacology Research Laboratory, Muhammad Institute, Multan. The experiments were performed according to the National Research Council guidelines[20] and approved by the Ethical Committee of Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan. They were maintained in clean, white polypropylene cages with specified relative humidity (40%–60%) and temperature (25°C ± 2°C) control. The natural 12 h light/dark cycle was maintained, and the animals were fed with standard pellet diet and had access to clean drinking water provided *ad libitum*.

Drugs and chemicals

Isoproterenol, formalin, ketamine, and xylazine purchased from prix lab lahore, ethanol; distilled water was purchased from M/s. Sigma Chemical Company, St. Louis. MO and kits obtained from Zell bio company, Germany, are used for research purposes. All the other chemicals used in the experiment were of analytical grade.

Extract concoction and preliminary phytochemical screening

J. mollissima plant had been collected from nearby Multan areas. It was authenticated by expert taxonomists' cooperation at the Department of Botany, Bahauddin Zakariya University, Multan. The voucher specimen (R. R. Stewart F. W. Pak. 704/11) has been placed for further reference. *J. mollissima* leaves were washed and powdered under a blender's shade for 15 days. In the experiment, the powder obtained from only one lot was used. For nine days, 800 g of *J. mollissima* leaves powder was soaked in a hydroalcoholic solvent (70:30 v/v) in 3 L colorful airtight amber pots. After filtering and filtration, it was evaporated by a rotary evaporator at reduced pressure and 30°C–40°C. The obtained semisolid residue was refrigerated before further analysis. The extract from *J. mollissima* leaves was used for the assessment of the cardioprotective activity against isoproterenol-induced cardiotoxicity.

Experimental design

Group 1 animals have been treated as checks. Animals in Group 2 were given isoproterenol (ISP) (100 mg/kg s. c.). Group 3 animals were fed regular medicine that is carvedilol (2 mg/kg). Group 4, 5, and 6 animals were supplied with various levels of *Jm. Cr* extracts such as 400, 600, and 800 mg, respectively, per kg body weight orally. All groups were subcutaneously injected with isoproterenol (100 mg/kg) on 22 and 23 days except group 1. Each group reported symptoms and mortality and was compared to that of group 2. The rats were slaughtered and autopsied after 48 h of the first administration of isoproterenol. Further biochemical and antioxidant properties' analysis was performed of the serum and tissue samples.

Blood samples and metabolic data collection

The animals were slaughtered, and blood samples were taken. In all experimental classes, blood was collected on the 24th day to calculate biochemical parameters. Rats were kept 24 h in metabolic cages and given free access to tap water. Similarly, on the 24th day, blood samples were collected in EDTA tubes. A regular centrifugation system was used to remove serum, and the samples were reserved at 30°C to estimate, and also kidneys were dissected out for evaluating *in vivo* antioxidant enzymes.

Estimation of electrocardiographing and *heart rate*

After 12 h of the second dose (isoproterenol), anesthetic conditions triggered by the combination of ketamine and xylazine in the experimental animals were observed to record the electrocardiographing (ECG) and the heart rate (Power Lab 8/35, AD Instruments, Australia). The data were obtained from Lab Chart Pro (AD Instruments, Australia).

Histopathology

Toward the end of the examination, a kidney from the animals in each group was analyzed under anesthesia for histopathological analysis. The heart was segregated, and then the ventricular part of the heart was rapidly moved to 10% formalin for histopathological evaluation. The tissue was soaked in paraffin. The section was cut to 5 µm in diphenyl xylene and stained with H and E.[23] Histopathological cardiac tissue changes were determined under a compound microscope, and microimages were taken.[24]

In vivo antioxidant activity

Rat kidneys at 10,000 rpm at 0°C were homogenized and centrifuged for 20 min. The supernatant is used to assess antioxidant enzyme levels using the spectrophotometer calorimetric process (Merck Thermo spectronic Model NO. UV-1, double beam). Glutathione reductase (GLR) is estimated by using the method described by,[25] Lipid peroxidation by (thiobarbituric acid reactive substances) method,[26] superoxide dismutase by,[27] and Catalase (CAT) by colorimetric assay.[28]

Statistical evaluation

Data were analyzed via one-way analytical variance and Bonferroni's all-mean *post hoc* test. It was indicated like a means ± standard error of mean, and methodological significance by different experimental groups was evaluated. Statistically relevant $P < 0.05$ values were taken into account.

RESULTS

Biochemical and antioxidant analyses had shown a substantial increase in the amount of serum labelling enzymes and antioxidant properties, including creatine kinase muscle-brain fraction (CK-MB), lactate dehydrogenase (LDH), triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and significant increases in heart and body weight alongside endogenous LPO levels in isoproterenol treated group in comparison with standard and extract-treated groups 2,3,4,5, and 6. Tables 1-6 show biochemical and antioxidant properties of the heart tissues of isoproterenol-induced and extract-treated groups. There was a very substantial rise ($P < 0.001$) in the amounts of LDH, triglycerides, SGOT, SGPT, and significant increases in heart and body weight in ISP and some cases, standard treated groups 2 For 24 consecutive days, Group 4 rats pretreated with the extract 400 mg/kg reported a mild decline in the biochemical and antioxidant parameters. For 24 days in a row, Group 5 rats pre-treated with extract 600 mg/kg observed a mild to moderate decrease in biochemical and antioxidant parameters. Group 6 rats pretreated significantly ($P < 0.001$) by extract dose of 800 mg/kg over 24 consecutive days prevented the effects of isoproterenol and preserved the biochemistry at an average level. The levels of superoxide dismutase (SPD), CAT, and GLR were significantly reduced. The treatment group level of parameters substantially increased the impact of MI caused by isoproterenol and in the standard group by taking them to normal values. The *Jm. Cr* ethanolic extract-treated groups have a heart defense against MI caused by ISP. The histopathological evaluation of the control group revealed an ordered sequence of cardiac myocytes with uncontained nuclei in the tissue [Figure 1]. The group of ISP displayed severe myofibrillary collagenous deterioration and interstitial tissue inflammation. The extract of *Jm* and standard medicinal carvedilol revealed that the phenotype with an intact nucleus and cardiovascular structure without fibrosis or cell-infiltration is basically in the normal range.

Table 1: Crude excerpt *Jatropha mollissima* and standard drug effect on body weight and heat weight

Group	Difference in BW (g)	Mean HW (g)
n	10.89±3.33	0.93±0.009
ISP	35.89±2.93[a]	1.2±0.020[a]
SD	20.18±5.09	0.91±0.009[b]
JM 400	7.91±5.15[b]	0.95±0.023[a, b, c]
JM 600	12.7±1.3[b]	0.84±0.008[a, b, c]
JM 800	9.2±2.1[b]	0.87±0.007[a, b, c]

[a]$P<0.05$ in relation to standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Data were analyzed with one-way ANOVA and then with multiple comparison tests after Tukey's. ANOVA: Analytical variance; SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; BW: Body weight; HW: Heat weight

Table 2: Gross extract *Jatropha mollissima* and standard drug effect on electrocardiographing

Group	ST (mV)	QT (s)	RR (ms)	HR (bpm)
n	0.44±0.03	0.22±0.014	0.18±0.004	360±6.8
ISP	−0.10±0.003[a]	0.28±0.009	0.13±0.003[a]	530±6.3[a]
SD	0.17±0.008[b]	0.24±0.008[b]	0.28±0.006[a,b]	319±2.4[a,b]
JM 400	0.06±0.005[a,b,c]	0.24±0.008	0.21±0.004[a,b,c]	408±4.4[a,b,c]
JM 600	0.04±0.004[a,b,c,d]	0.20±0.014	0.22±0.004[a,b,c]	389±2.9[a,b,c]
JM 800	0.09±0.005[a,b,c,d,e]	0.20±0.013[b]	0.27±0.007[a,b,d,e]	341±5.41[b,d,e]

[a]$P<0.05$ in conjunction with standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Results were examined with one-way ANOVA and then with multiple comparison tests after Tukey's. SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; ANOVA: Analytical variance; ST: segment; HR: Heart rate

Table 3: The effect of standard drug and *Jatropha mollissima* crude extract in rats treated with isoproterenol on serum parameters

Group	CKMB	LDH
n	16.93±0.51	104.77±0.60
ISP	36.22±0.77[a]	142.88±0.60[a]
SD	20.90±0.84[a,b]	122.69±0.97[a,b]
JM 400	29.96±0.87[a,b,d]	138.52±0.97[a,b,c,d]
JM 600	25.74±0.85[a,b,c]	133.90±1.22[b]
JM 800	22.86±0.90[a,b,c]	125.44±0

[a]$P<0.05$ in relation to standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Results were examined with one-way ANOVA and then with multiple comparison tests after Tukey's. SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; LDH: Lactate dehydrogenase; CKMB: Creatine kinase muscle-brain; ANOVA: Analytical variance

DISCUSSION

Heart disease is a worldwide health epidemic. The administrative drain is motivated by both the mortality rate and the risk factors. CVD was expected to rank 1st from 1990 to 2020.[5] In the current research, ISP mediated detrimental heart damage in rats, confirmed by biochemical

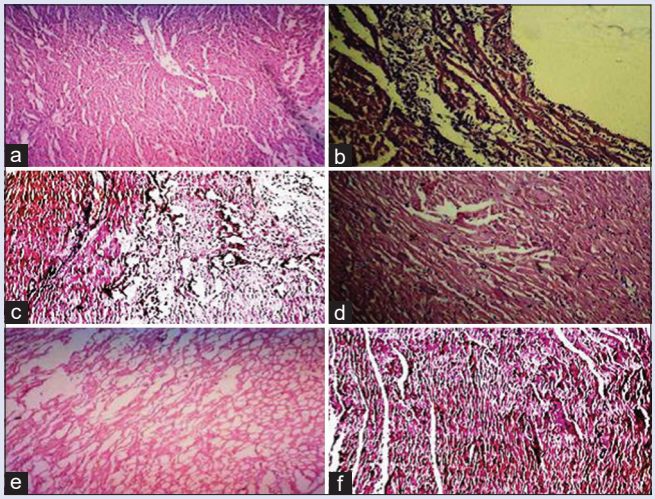

Figure 1: Histopathological sections of the rat kidney; (a) Control (b) ISP (c) SD (d) ISP + JM (400 mg/kg) (e) ISP + JM (600 mg/kg) (f) ISP + JM (800 mg/kg). ISP: Isoproterenol; SD: Standard drug; JM: *Jatropha mollissima*

assessments and histopathological analysis. Cardiotoxicity caused by ISP offers vast information into this disease, which strongly demonstrates lipid peroxidation involvement.[28]

ISP is an artificial neurotransmitter, and β-agonist induces extreme ventricular oxidative stress and fibrosis of cardiac tissues that progress to MI progression. The most validated form of MI in rats is the ISP ventricular edema.[29] ISP creates extremely cytotoxic reactive oxygen species known as membrane phospholipid peroxidative damage, which poses a significant threat to the microvascular envelope during autoxidation.[30]

J. mollissima is widely used in folk medicine for different purposes. Its natural latex shows antimicrobial activity[31] and can be used for snakebites and healing injuries.[32] The seeds of the plant are used for relaxing and antidepressants.[4] The leaves are antioxidants for boosting appetite[3] and are applied in infections of the kidney,[33] whereas the stalk shows an anthelmintic effect.[34]

This study showed that ISP administration in male Wistar rats causes acute MI with a dose of 100 mg/kg for 2 days in a row.

The results of this study showed that ISP administration in male Wistar rats was acute MI with 100 mg/kg for 2 days in a row. Evolving patterns of ECG irregularities are the key requirement for the definite diagnosis of MI. ISP administration substantially decreased the P wave and QRS complex and elevated the ST portion. It was stated in previous studies that administration of ISP 100 mg/kg in male standard drug (SD) rats increases the segments ST, QT and lower P wave, RR interval and QRS complex in two consecutive days.[26] Various studies have also shown that ST-segment elevation and agitation are characteristic of the AMI model in ISP-induced rats. The change in the ECG parameter is caused by changes in the possible membrane in the infarction region.[26,27] In contrast to the treatments of isoproterenol, the section ST, QT interval, RR interval and core rate of the animals treated with JM extract 400, 600 and 800 mg/kg showed important improvements. QT interval and cardiac rate have been shown to be significantly increased in the ISP treatment community, whereas the JM extract treated groups have decreased due to decreased myocardial injury. The RR interval was decreased for the treatment group with isoproterenol while the treated groups with the JM extract showed higher intervals. The cardiac concentrations in the carvedilol group showed better results compared to the *J. mollissima* groups treated [Figure 2 and Table 1]. Changes in these parameters

Table 4: *Jatropha mollissima* crude extract and standard drug impact on serum confines in rats treated with isoproterenol

Group	TG	SGOT
n	71.20±0.50	54.77±0.20
ISP	77.25±0.80[a]	96.45±0.24[a]
SD	69.40±0.76[a,b]	57.80±0.28[a,b]
JM 400	68.77±0.74[a,b,c,d,e]	64.18±0.42[a,b,c]
JM 600	65.99±0.86[a,b]	61.52±0.49[a,b,c,d]
JM 800	62.47±0.68[a,b,c,d]	59.95±0.43[a,b,c,d]

[a]$P<0.05$ in conjunction with standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Data were analyzed with one-way ANOVA and then with multiple comparison tests after Tukey's. SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; TG: Triglyceride; SGOT: Serum glutamic oxaloacetic transaminase; ANOVA: Analytical variance

Table 5: Gross extract *Jatropha mollissima* and standard drug effect on isoproterenol-treated rats' serum confines

Group	SGPT	TNT
n	35.90±0.55	Negative
ISP	58.64±0.57[a]	Positive
SD	39.33±0.40[a,b]	Negative
JM 400	50.55±0.53[b,c]	Negative
JM 600	47.85±0.53[a,b,c,d]	Negative
JM 800	44.24±0.48[a,b,c,d,e]	Negative

[a]$P<0.05$ in conjunction with standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Data were analyzed with one-way ANOVA and then with multiple comparison tests after Tukey's. SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; SGPT: Serum glutamic pyruvic transaminase; TNT: Trinitrotoluene; ANOVA: Analytical variance

Table 6: Crude *Jatropha mollissima* extract and standard drug effect in rats treated with isoproterenol on antioxidant properties of enzymes

Group	SPD (U/mg)	CAT (U/mg)
n	12.57±0.08	8.70±0.84
ISP	8.35±0.03[a]	2.53±0.42[a]
SD	10.99±0.04[a,b]	10.23±0.57[b]
JM 400	9.99±0.261[b,c]	6.43±0.42[b,c]
JM 600	10.46±0.42[a,b,d]	6.02±0.55[b,c]
JM 800	10.07±0.26[b,d]	7.45±0.57[b,c]

[a]$P<0.05$ in conjunction with standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Data were analyzed with one-way ANOVA and then with multiple comparison tests after Tukey's. SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; SPD: Superoxide dismutase; CAT: Catalase; ANOVA: Analytical variance

revealed that isoproterenol-treated animals had experienced myocardial infection and the JM extract administered groups exhibited substantial reductions in the elevation of the stiffness section which may suggest the reverse effects of treatment with isoproterenol. Previous study showed that escalation of the ST segment culminated in depletion of membrane feature representing the possible disparity between hemorrhagic and non-ischemic areas.[28]

The CK-MB activity, LDH, triglycerides, SGOT, and SGPT enzymes were significantly increased in the group treated with isoproterenol than the control group showed substantial improvements in treated SGOT, SGPT groups compared with *J. mollissima* extract. Compared with the isoproterenol treated group, the treatment groups showed a significant

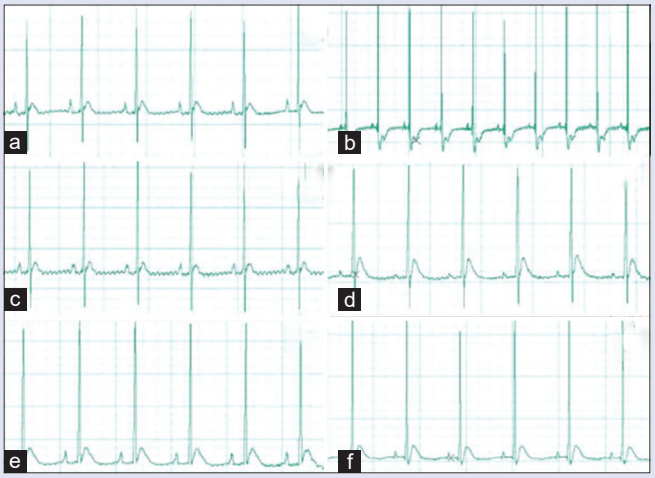

Figure 2: ECG of normal and cured groups. (a) N, (b) ISP, (c) SD, (d) JM400, (e) JM600, (f) JM800. ISP: Isoproterenol; SD: Standard drug; JM: *Jatropha mollissima*; ECG: Electrocardiographing; N: Normal

decrease in enzyme levels. Triglycerides were found to be substantially decreased compared to the control group, stages. As sensitive markers of heart injury, substantial changes in the serum biochemical enzyme levels are used. The increase in serum biomarkers indicates that the mitochondrial enzymes are leaked due to myocardial injury.[9] It shows that the level of heart myocardial injury was considerably lower in pretreated classes. The cardioprotective function of the test drugs is thus demonstrated.

Troponin T is a cardiac regulating protein that controls muscle, actin, and myosin-mediated interactions, resulting in expansion and contentment of muscle fibers. Troponin T is unique to the myocardium. Insufficient blood flow and oxygen supply to the myocardium induce necrosis and eventual release of 2,4,6-trinitrotoluene and troponin I into the bloodstream. Just ISP treated group showed positive for Trop-T in serum, while the control, standard, and *J. mollissima* extract pre-treated groups showed adverse outcomes. The increased activity of serum Trop T noticed in isoproterenol-induced rats was due to ISP's cardiac damage.[35]

LPO is a known cellular injury mechanism and was seen as an antioxidant predictor strain leading towards MI pathogenicity.[3] In ISP-treated myocardial rats, cardiac trauma triggered by oxidation products has been documented. The myocardial necrosis found in ISP recipient rats may be caused by peroxidative damage because ISP produces lipid peroxides.[36] For myocardial cells, the accumulation of lipid peroxides in free radicals contributes to increased LPO thresholds in the cardiomyocytes. The LPO levels of the isoproterenol mediated group have increased significantly while Jm. Cr pretreated groups reduced LPO levels dramatically as shown in Table 7. The normal carvedilol inhibited the rise of LPO significantly. These results indicate that test drugs' pretreatment groups substantially impact LPO inhibition, reducing oxidative stress and retaining membranous integrity. The group induced by isoproterenol showed a substantial decrease in SPD, CAT, and GLR relative to the control group. Pretreated classes compared to the control group, carvedilol, *J. mollissima* extract, significantly raised levels. It shows that the test medication substantially influences the oxidative stress reduction caused by free radicals. The decreases in these enzymes' activity can be attributed to their enhanced use of Reactive oxygen species and their scavenging excessive ISP oxidation inactivation.[37]

Table 7: Crude *Jatropha mollissima* extract and standard drug effect in rats treated with isoproterenol on antioxidant properties of enzymes

Group	GLR	LPO (MDA n moles/mg protein)
n	8.02±0.84	0.485±0.046
ISP	2.53±0.42[a]	0.663±0.005[a]
SD	10.23±0.57[b]	0.582±0.037[b]
JM 400	6.43±0.42[b]	0.536±0.022[a]
JM 600	6.02±0.55[b, c]	0.620±0.004[a,b]
JM 800	7.45±0.57[b,c,d,e]	0.545±0.006[b,d,e]

[a]$P<0.05$ in conjunction with standard; [b]$P<0.05$ in relation to negative control; [c]$P<0.05$ in relation to positive control; [d]$P<0.05$ in relation to JM 400; [e]$P<0.05$ in relation to JM 600. All values have mean±SEM, $n=6$. Data were analyzed with one-way ANOVA and then with multiple comparison tests after Tukey's. SEM: Standard error of mean; ISP: Isoproterenol; JM: *Jatropha mollissima*; SD: Standard drug; GLR: Glutathione reductase; LPO: Lipid peroxidation; ANOVA: Analytical variance; MDA: Malondialdehyde

We suggest that the plant extract contains constituents with cardioprotective and cancer prevention agents acting as potent freelance radicals scavengers in the heart to counteract the lethal impacts of ISP. So my study was designed to determine the cardioprotective action of *J. mollissima* extract against ISP induced cardiotoxicity.

An organic pattern of myocardial cells with intact nuclei in tissue has been seen in the control group, and it was observed that the cardiac morphology is regular [Figure 2]. The category of isoproterenol has demonstrated severe myofibrillar myxoid degeneration and myocardial tissue interstitial edema. The elicit of *J. mollissima* treated groups and the regular medicine carvedilol showed that the Myocardial levels' morphology was mainly within normal limits with an intact nucleus and cardiovascular morphology, no cellulitis. Comparable GA results with high antioxidant properties were identified in ISP-treated rats.[9,35] Finding the exact mechanics of action needs more study. It is still controversial whether isoproterenol cardiotoxicity is cumulative. This finding is consistent with the result of other researchers. This study's results showed that isoproterenol administration had caused myocardial damage, as evidenced by increasing and decreasing concentrations of biochemical and antioxidant properties and histopathological changes in cardiac myocytes associated with free radical development. The theoretical gap for *J. mollissima* isolation is left behind in this report.

CONCLUSION

Ethanolic leaves extract of *J. mollissima* secured the experimentally induced MI in rats as shown by the amelioration of histopathological variations, biochemical of cardiac tissue injury. Further studies are recommended to correct the precise mode of action for intervention and determine the exact cardioprotective potential of phytoconstituents responsible for it.

Acknowledgements

We are grateful to the laboratory staff and staff of the animal house of Muhammad Institute of Medical, Allied Sciences, Multan, for their cooperation throughout the study and Shifa Clinical laboratory Multan to provide research facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nestelberger T, Boeddinghaus J, Wussler D, Twerenbold R, Badertscher P, Wildi K, *et al.* Predicting major adverse events in patients with acute myocardial infarction. J Am Coll Cardiol 2019;74:842-54.
2. Boudina S, Laclau MN, Tariosse L, Daret D, Gouverneur G, Bonoron-Adèle S, *et al.* Alteration of mitochondrial function in a model of chronic ischemia *in vivo* in rat heart. Am J Physiol Heart Circ Physiol 2002;282:H821-31.
3. Mohanty I, Arya DS, Dinda A, Talwar KK, Joshi S, Gupta SK. Mechanisms of cardioprotective effect of Withania somnifera in experimentally induced myocardial infarction. Basic Clin Pharmacol Toxicol 2004;94:184-90.
4. Sabeena Farvin KH, Anandan R, Kumar SH, Shiny KS, Sankar TV, Thankappan TK. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. Pharmacol Res 2004;50:231-6.
5. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997;349:1498-504.
6. Bloom S, Cancilla PA. Myocytolysis and mitochondrial calcification in rat myocardium after low doses of isoproterenol. Am J Pathol 1969;54:373-91.
7. Rona G. Catecholamine cardiotoxicity. J Mol Cell Cardiol 1985;17:291-306.
8. Bartekova M, Ferenczyova K, Jelemensky M, Dhalla NS. Role of oxidative stress and cardiovascular risk factors in ischemic heart disease. In: Oxidative Stress in Heart Diseases. Singapore: Springer; 2019. p. 375-94.
9. Priscilla DH, Prince PS. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem Biol Interact 2009;179:118-24.
10. Mathew TH. Drug-induced renal disease. Med J Aust 1992;156:724-9.
11. Topliss JG, Clark AM, Ernst E, Hufford CD, Johnston GA, Rimoldi JM, *et al.* Natural and synthetic substances related to human health (IUPAC Technical Report). Pure Appl Chem 2002;74:1957-85.
12. Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Di Paola R, Britti D, *et al.* A role for superoxide in gentamicin-mediated nephropathy in rats. Eur J Pharmacol 2002;450:67-76.
13. Guidet BR, Shah SV. *In vivo* generation of hydrogen peroxide by rat kidney cortex and glomeruli. Am J Physiol Renal Physiol 1989;256:F158-64.
14. Yang CL, Du XH, Han Y×. Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. Ren Fail 1995;17:21-6.
15. Parlakpinar H, Tasdemir S, Polat A, Bay-Karabulut A, Vardi N, Ucar M, *et al.* Protective role of caffeic acid phenethyl ester (cape) on gentamicin-induced acute renal toxicity in rats. Toxicology 2005;207:169-77.
16. Gomes JA, Félix-Silva J, Morais Fernandes J, Geraldo Amaral J, Lopes NP, Tabosa do Egito ES, *et al.* Aqueous leaf extract of *Jatropha mollissima* (Pohl) bail decreases local effects induced by bothropic venom. Biomed Res Int 2016;2016:6101742.
17. Longoni B, Migliori M, Ferretti A, Origlia N, Panichi V, Boggi U, *et al.* Melatonin prevents cyclosporine-induced nephrotoxicity in isolated and perfused rat kidney. Free Radic Res 2002;36:357-63.
18. Piotrowski WJ, Pietras T, Kurmanowska Z, Nowak D, Marczak J, Marks-Kończalik J, *et al.* Effect of paraquat intoxication and ambroxol treatment on hydrogen peroxide production and lipid peroxidation in selected organs of rat. J Appl Toxicol 1996;16:501-7.
19. Priuska EM, Schacht J. Formation of free radicals by gentamicin and iron and evidence for an iron/gentamicin complex. Biochem Pharmacol 1995;50:1749-52.
20. Wiland P, Szechciński J. Proximal tubule damage in patients treated with gentamicin or amikacin. Pol J Pharmacol 2003;55:631-7.
21. Iqbal MO, Yahya EB. *In vivo* assessment of reversing aminoglycoside antibiotics nephrotoxicity using *Jatropha mollissima* crude extract. Tissue Cell 2021;72:101525.
22. Bastos-Cavalcante N, dos Santos-Barbosa CR, Silva-Pereira RL, Feitosa-Muniz D, de Melo-Coutinho HD, Araújo-Rolim L, *et al.* Phytochemical analysis, antibacterial activity and antibiotic modifying action of *Jatropha mollissima* (Pohl.) Baill. (Euphorbiaceae). Servicio de Publicaciones de la Universidad de Murcia. In Anales de Biología 2020;42:85-94.
23. Yanagida C, Ito K, Komiya I, Horie T. Protective effect of fosfomycin on gentamicin-induced lipid peroxidation of rat renal tissue. Chem Biol Interact 2004;148:139-47.
24. Kumar V, Babu V, Nagarajan K, Machawal L, Bajaj U. Protective effects of centella asiatica against isoproterenol-induced myocardial infarction in rats: Biochemical, mitochondrial and histological findings. J Phytopharmacol 2015;4:80-6.
25. Panda VS, Naik SR. Cardioprotective activity of *Ginkgo biloba* Phytosomes in isoproterenol-induced myocardial necrosis in rats: A biochemical and histoarchitectural evaluation. Exp Toxicol Pathol 2008;60:397-404.
26. Shaik AH, Rasool SN, Vikram Kumar Reddy A, Abdul Kareem M, Saayi Krushna G, Lakshmi Devi K. Cardioprotective effect of HPLC standardized ethanolic extract of *Terminalia pallida* fruits against isoproterenol-induced myocardial infarction in albino rats. J Ethnopharmacol 2012;141:33-40.
27. Panda VS, N SR. Evaluation of cardioprotective activity of *Ginkgo biloba* and *Ocimum sanctum* in rodents. Altern Med Rev 2009;14:161-71.
28. Upaganlawar A, Balaraman R. Cardioprotective effects of *Lagenaria siceraria* fruit juice on isoproterenol-induced myocardial infarction in wistar rats: A biochemical and histoarchitecture study. J Young Pharm 2011;3:297-303.
29. Patel V, Upaganlawar A, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation. Eur J Pharmacol 2010;644:160-8.
30. Aman U, Vaibhav P, Balaraman R. Tomato lycopene attenuates myocardial infarction induced by isoproterenol: Electrocardiographic, biochemical and anti-apoptotic study. Asian Pac J Trop Biomed 2012;2:345-51.
31. Diercks DB, Roe MT, Chen AY, Peacock WF, Kirk JD, Pollack CV Jr., *et al.* Prolonged emergency department stays of non-ST-segment-elevation myocardial infarction patients are associated with worse adherence to the American College of Cardiology/American Heart Association guidelines for management and increased adverse events. Ann Emerg Med 2007;50:489-96.
32. Bahit MC, Murphy SA, Gibson MC, Cannon CP. Critical pathway for acute ST-segment elevation myocardial infarction: Estimating its potential impact in the TIMI 9 Registry. Crit Pathw Cardiol 2002;1:107-12.
33. Blasig IE, Blasig R, Löwe H. Myocardial lipid peroxidation during isoproterenol-induced blood flow reduction in rat myocardium. Biomed Biochim Acta 1984;43:S171-4.
34. Saravanan G, Ponmurugan P, Sathiyavathi M, Vadivukkarasi S, Sengottuvelu S. Cardioprotective activity of *Amaranthus viridis* Linn: Effect on serum marker enzymes, cardiac troponin and antioxidant system in experimental myocardial infarcted rats. Int J Cardiol 2013;165:494-8.
35. Walter LS, Gabira MM, da Silva MA, Nogueira AC, Kratz D. Adjustments in the tetrazolium test methodology for assessing the physiological quality of *Jatropha mollissima* (Euphorbiaceae). Bosque 2020;41:77-82.
36. Walter LS, Gabira MM, Almeida da Silva M, Nogueira AC, Kratz D. Adecuación metodológica de la prueba de tetrazolio para semillas de *Jatropha mollissima* (Euphorbiaceae). Bosque (Valdivia) 2020;41:77-82.
37. Araújo JR, da Silva Costa MW, Oliveira WB, Cavalcante RR, Almeida PM, Martins FA. Larvicidal, cytotoxic and genotoxic effects of aqueous leaf extract of *Jatropha mollissima* (Pohl) Baill. Acta Sci Biol Sci 2018;40:e34501.