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Original Article

CARDIOPROTECTIVE EFFECT OF *TAMARINDUS INDICA LINN* AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

Objective: The aim of the current study was to evaluate the cardioprotective activity of *Tamarindus Indica. Linn* in Isoproterenol hydrochloride-induced Myocardial Infarction in rats.

Methods: *In vitro* antioxidant activity of aqueous fruit extract (AQFE), alcoholic fruit extract (ALFE), aqueous seed extract (AQSE) and alcoholic seed extract (ALSE) of *Tamarindus indica* Linn (TI) was carried by well-established *in vitro* methods. The extracts were investigated for *in vivo* cardioprotective activity against IPRT induced myocardial infarction where the animals were treated with the extracts (250 mg and 500 mg/kg body weight) for 30 d. Standard vitamin E (100 mg/kg body weight) was used to compare the effect of the extracts. Change in body weight, ECG, heart weight, serum parameters, myocardial infarct area and enzyme estimation in heart homogenate were assessed.

Results: ALSE, AQSE and ALFE showed potent antioxidant activity with an IC_{50} of 12.94±0.14, 252.49±0.47 and 647.39±1.15 µg/ml. ALSE 250 mg and 500 mg showed dose-dependent cardioprotective activity in cardiotoxic rats. IPRT treated animals showed a marked increase in serum marker enzymes, an increase in the percent infarction area increase in heart weight and a decrease in body weight along with the decrease in endogenous enzyme levels were observed.

Conclusion: The administered extracts significantly reversed the effects caused by IPRT. ALSE 250 and 500 mg/kg body weight showed maximum reversal while the other extracts AQSE, ALFE and AQFE were observed to have good antioxidant activity *in vitro*.

Keywords: Isoproterenol, *Tamarindus indica. Linn*, Antioxidant, Myocardial infarction, Electrocardiography, Cardiac markers

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INTRODUCTION

Cardiovascular diseases (CVDs) are the number one cause of death globally [1] The number of people, who die from CVDs, mainly from heart disease and stroke, will increase to reach 23.3 million by 2030 [2]. In India heart diseases have emerged as the number one killer in both urban and rural areas of the country. Presently 1.2 billion people in India are suffering from heart disease [3].

There is a continued interest in developing therapeutics to combat human injury sustained from a myocardial infarction. To date, the therapy for acute ischemia of the heart has been largely directed towards re-establishing perfusion of the ischemic myocardium or towards the coagulation system to prevent thrombosis. Therefore, strategies that would improve treatment of different forms of myocardial ischemia are reasonable.

In past 20 y, it has been recognised that there are endogenous mechanisms that, when activated, protect the heart against ischemia, and they have been described as a mechanism of cardioprotection where ROS (Reactive Oxygen Species) is one of the key factors. ROS can cause oxidative damage to a variety of cellular components, and it displays a significant role in the etiology of myocardial ischaemia-reperfusion (I/R) injury [4]. Interest in the search for new natural antioxidants has grown over the past years because ROS production and oxidative stress have been shown to be linked to cardiovascular disease.

Natural antioxidants are believed to play a potential role in interfering with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen in biological systems [5]. The use of traditional medicine is widespread and herbals still present a large source of novel active biological compounds with different activities.

Tamarindus indica. Linn (TI) seed and fruit pulp is widely used in traditional food, folklore medicine, and ayurvedic herbal treatment and has been subjected to various experimental activities which have proven it to be a potent antioxidant. However, there is no substantial evidence to link the use of this herbal formulation as a cardioprotective agent. An effort is put up to reveal whether the pre and co-treatment with the seed and fruit extract of TI yields a substantial change in myocardial injury.

Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] (IPRT) a synthetic catecholamine and a β-adrenergic agonist, has been established to produce extreme stress in the myocardium leading to myocardial infarction, and when administered in supramaximal doses [6,7] it leads to myocardial necrosis which roots to cardiac dysfunction, increased lipid peroxidation besides an elevated level of myocardial lipids as well as altered activities of the cardiac enzymes and antioxidants [8]. The present study was designed to study the result of TI pre-treatment on the myocardial infarction brought about by supramaximal doses of IPRT. The existing study also endeavoured to establish the possible mechanism of its therapeutic efficacy by studying the biochemical markers, antioxidant defence system, lipid profile, electrocardiographic and histopathological changes.

MATERIALS AND METHODS

Drugs and chemicals

Isoproterenol hydrochloride, nitro blue tetrazolium (NBT), phenazine methosulphate, butylated hydroxytoluene, 1-chloro-2,4-dinitro benzene, 2,4-dinitro phenyl hydrazine, p-phenylene diamine, reduced glutathione and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co., Bommasandra-Jigani Link Road, Bengaluru-560100, Karnataka, India. Thiobarbituric acid and trichloroacetic acid were purchased from Hi-media, Mumbai, India. All the other chemicals and reagents used were of analytical grade

Collection of plants

The plant material fresh fruits and seeds of TI was procured from local market. The pericarp of the fruits was peeled, and the adhering dust was removed. The seeds were dried in hot air oven at 40 ºC for 24 h and were powdered using a mechanical grinder.

Preparation of extract

The coarse powder of the seeds and small pieces of the fruit pulp were then subjected for extraction by cold maceration technique separately. After four days the aqueous and alcoholic macerate were decanted and pressed to extract the solvent which was then kept in separate beakers that were covered using cling film to avoid contamination and stored away from sunlight while the marc was replenished with fresh solvents.

This process continued for five cycles. The aqueous macerate was concentrated by allowing it to settle and then decant the excess aqueous layer which was then evaporated using steam over a water bath in large petri dishes. The alcoholic macerate was concentrated by distilling out the alcohol and further dried using steam in petri dishes.

***In vitro* antioxidant study**

Scavenging of free radical DPPH

The ability to scavenge DPPH stable free radical by all the extracts in a concentration range of 20 to 100 µg/ml was determined [9].

Scavenging of free ABTS radical

The ability to scavenge the stable free ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical by all the extracts in a concentration range of 20 to 100 µg/ml was determined [10].

Superoxide anion radical scavenging activity

The ability to scavenge the hydroxyl radicals as well as singlet oxygen by all the extracts in a concentration range of 20 to 100 µg/ml was determined [11].

Animals

Albino Wistar rats weighing 180–200 g were procured from the Central Animal House, JSS Medical College, JSS University, Karnataka, for the present study. They were housed in polypropylene cages with paddy husk as bedding and maintained in standard condition (12 h light and dark cycle, 24 °C.). The rats had free access to drinking water and standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India) ad libitum.

The experiment was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and the present study was approved by the Institutional Animal Ethical Committee of JSS College of Pharmacy, Mysore (Proposal No: 135/2013).

Experimental design

After acclimatization, the animals were randomly divided into following groups consisting of 8 rats each:

Group 1: Normal (Saline 1 ml/rat per oral (p. o))

Group 2: Negative Control-IPRT (100 mg/kg) 29th and 30th day

Group 3: Positive Control-Vitamin E (100 mg/kg p. o)

Group 4: Alcoholic Seed Extract (ALSE) (250 mg/kg p. o)

Group 5: ALSE (500 mg/kg p. o)

Group 6: Aqueous Seed Extract (AQSE) (250 mg/kg p. o)

Group 7: AQSE (500 mg/kg p. o)

Group 8: Alcoholic Fruit Extract (ALFE) (250 mg/kg p. o)

Group 9: ALFE (500 mg/kg p. o)

All the animals in the group 4 to 9 were treated with their respective extracts for 30 d. Group 3 received standard drug Vitamin E. On 29th and 30th d of the treatment all groups except Normal group were injected with 100 mg/kg, subcutaneous (s. c) of IPRT in saline (final volume of 0.25 ml) to induce myocardial infarction.

***In vivo* cardioprotective activity**

Induction of experimental myocardial infarction

IPRT (100 mg/kg body weight) dissolved in saline and was injected subcutaneously to rats at an interval of 24 h for two days to induce myocardial infarction [12]. Animals were sacrificed 48 h after the first dose of IPRT.

Differences in body weight

The change in body weight after treatment was recorded every day before dosing and percentage reduction or increase in body weight was reported [13].

Differences in heart weight

After Electrocardiography (ECG) recordings, animals were euthanized to isolate the heart and the heart wet weight of all the experimental animals was measured [13].

Electrocardiography

The ECG patterns were recorded by 3 lead-2-channel polygraph (INCO polygraph). ECG recordings were performed in anesthetized rats. The changes in ST-segment elevation, QT interval, RR interval and heart rate were recorded in control, normal and all the groups of treated animals.

Estimation of myocardial damage by triphenyl tetrazolium (TTC) assay method

TTC assay was performed as per the protocol [14,15]. In brief, the heart was frozen immediately after removal. When the tissue was firm, the heart was transversely cut across the left ventricle and sections of 1–2 mm thick were incubated in 1% TTC solution prepared in phosphate buffer (pH 7.4) for 30 min at 37 °C. At the end of the incubation period, the heart slices were kept in fixing solution to fix the tissue. A Camera with Macro lens was used to take photographs and the percentage infarction was measured using mobile infarct area software.

Biochemical analysis

On the 31st day of the study, blood was collected by cardiac puncture. The blood was allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 8000 rpm for 10 min. The collected serum was used for the estimation of cardiac marker enzymes Creatine Kinase-Muscle/Brain Isoenzyme (CK-MB), lactate dehydrogenase (LDH), Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) using commercially available enzymatic kits (Span Diagnostics Pvt. Ltd., India). Serum was also estimated for the lipid profile including total cholesterol and triglycerides using along with Total protein commercially available kits (Span Diagnostics Pvt. Ltd., India) [16,17].

Assay of lipid peroxidation products and antioxidant systems

Thiobarbituric acid reactive substances in the heart tissue homogenate were estimated [18]. The activities of superoxide dismutase, catalase and glutathione reductase in the heart tissue homogenates were assayed by the standard methods [19-22] respectively. The level of reduced glutathione in the heart tissue homogenate was estimated [23]. The protein content in the heart tissue homogenate was determined [24].

Statistical analysis

Data was expressed as mean±SEM for eight rats. Statistical analysis was performed by one-way analysis of variance followed by Tukey's multiple comparison tests using software Graph Pad Prism Version 5.0. p values<0.05 were considered significant.

RESULTS

***In vitro* antioxidant study**

The percentage scavenging effects of ALSE, AQSE, ALFE and AQFE were carried out using various well established *in vitro* assays like DPPH scavenging assay, ABTS scavenging assay and Superoxide

anion radical scavenging activity. All the extracts scavenged these free radicals in a dose dependent manner. ALSE showed most potent scavenging activity among the four extracts used, with an IC_{50} value of 12.94±0.14, 45.86±0.08 and 202.63±0.21 µg/ml in DPPH, ABTS and superoxide anion radical assay respectively, when compared with the standard Ascorbic acid where the IC_{50} for the above-mentioned assays are 4.46±0.60, 22.56±1.29 and 59.54±0.44 µg/ml.

Based on these results obtained and when compared with the other extracts AQFE was rejected since it was not significant to the IC_{50} values of the other extracts and hence a decreased potency in its ability to scavenge free radicals.

Thus, ALSE, AQSE and ALFE were further in the *in vivo* studies.

In vivo cardioprotective activity

Effect of ALSE, AQSE and ALFE on bodyweight

Body weight at the end of the experiment in Negative Control group was significantly increased ($p<0.05$) when compared to the Normal Group, whereas in treatment groups there was a significant decrease ($p<0.05$) in the body weight as shown in fig. 1.

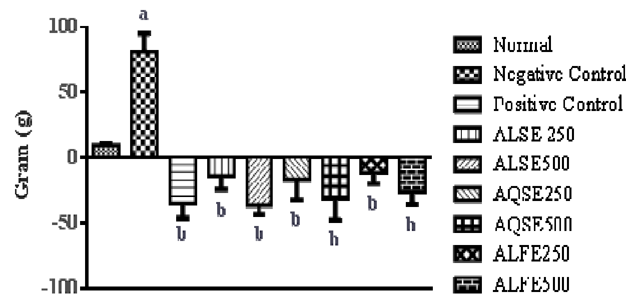

Fig. 1: Effect of ALSE, AQSE and ALFE on bodyweight

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at [a]$p<0.05$ when compared to Normal, [b]$p<0.05$ when compared to Negative control, [c]$p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison tests.

ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract

Effect of ALSE, AQSE and ALFE on heart weight

There was a significant increase ($p<0.05$) in heart weight of the negative control group when compared to the normal group. A significant decrease ($p<0.05$) in heart weight was seen in treatment groups as shown in fig. 2.

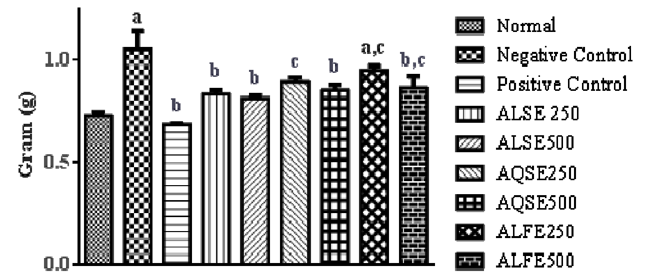

Fig. 2: Effect of ALSE, AQSE and ALFE on heart weight

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at [a]$p<0.05$ when compared to Normal, [b]$p<0.05$ when compared to Negative control,[c]$p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison tests.

ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract

Electrocardiography

The ECG parameters of all the groups except normal revealed a significant increase ($p<0.05$) in heart rate, ST segment elevation and QT interval while a decreased ($p<0.05$) RR interval was seen when compared with normal. These changes are almost similar to the indications of MI. Pre-and co-treatment with the various extracts decreased ($p<0.05$) the heart rate, ST segment elevation, and QT interval, while an increased ($p<0.05$) RR interval was seen when compared with the negative control group.

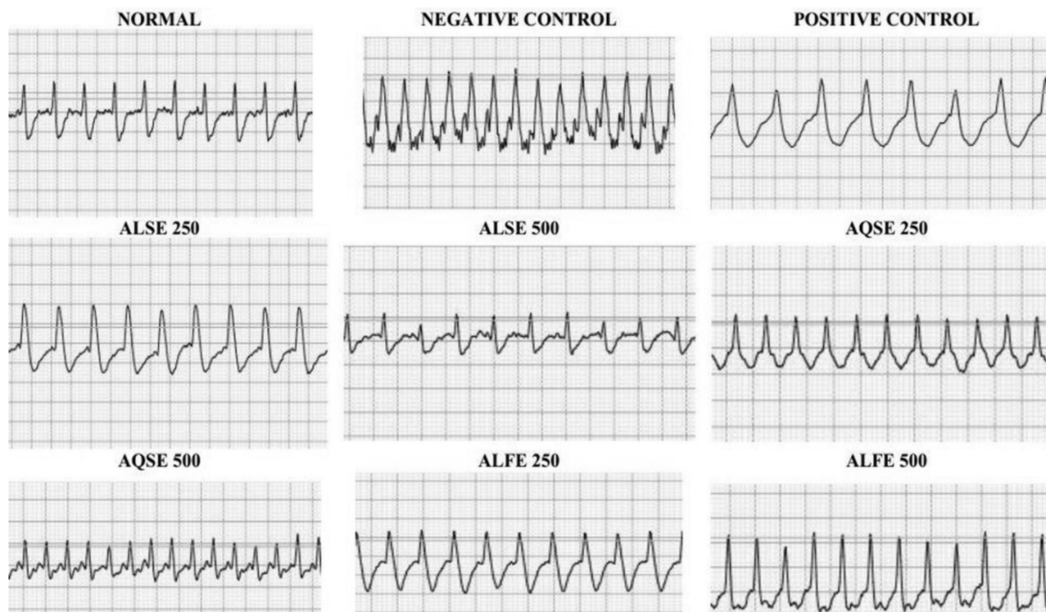

Fig. 3: Effect of ALSE, AQSE and ALFE pre-treatment on electrocardiographic patterns in isoproterenol-induced myocardial infarction in rats

ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract

Table 1: Effect of ALSE, AQSE and ALFE pre-treatment on electrocardiographic parameters in isoproterenol-induced myocardial infarction in rats

Groups	ST mV	QT mSec	RR mSec	HR BPM
Normal	0.03±0.06	395.75±14.17	252±3.87	374.12±1.12
Negative Control	0.08±0.01[a]	560.25±10.82[a]	201.12±6.67[a]	468.37±1.88[a]
Positive Control	0.04±0.03[b]	402.5±8.12	256.62±8.60[b]	376.37±0.77[b]
ALSE 250	0.05±0.02[b]	458.5±2.35[a,b,c]	250.75±5.35[b]	392.37±0.73[a,b,c]
ALSE500	0.05±0.02[b]	425±1.10[b]	247.25±12.01[b]	342.87±0.93[a,b,c]
AQSE250	0.04±0.03[b]	472.5±3.47[a,b,c]	251.25±10.12[b]	393.12±1.05[a,b,c]
AQSE500	0.05±0.04[b]	455.87±3.34[a,b,c]	250.125±1.81[b]	382.50±0.62[a,b,c]
ALFE250	0.05±0.05[b]	462±1.41[a,b,c]	254±13.03[b]	412.87±2.12[a,b,c]
ALFE500	0.05±0.07[b]	443±2.63[a,b,c]	252.62±5.93[b]	394.25±0.95[a,b,c]

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at, [a]$p<0.05$ when compared to Normal, [b]$p<0.05$ when compared to Negative control, [c]$p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison tests.

ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract; HR-Heart Rate

Estimation of myocardial damage by triphenyl tetrazolium (TTC) assay method

The area of necrosis was determined by TTC staining as shown in fig. 5. The heart section from normal animals revealed completely viable myocardial tissue stained with TTC to indicate the presence of LDH and intact myocardial tissue. The heart sections from negative control group indicated a large unstained area.

However, the heart sections of animals which were pre-and co-treated with various extracts exhibited major portion stained, showing tissue viability with less necrotic tissues. The percentage area at risk for necrosis was assessed which revealed that negative control showed an increased ($p<0.05$) risk when compared with the treatment groups as shown in fig. 4.

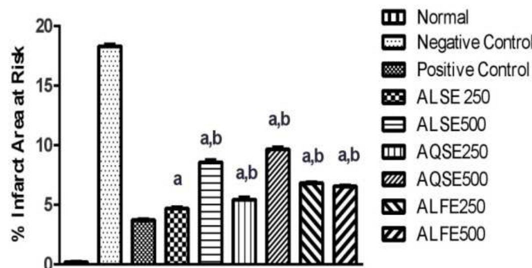

Fig. 4: Effect of ALSE, AQSE and ALFE pre-treatment on myocardial infarct area in isoproterenol-induced myocardial infarction in rats.

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at [a]$p<0.05$ when compared to Normal, [b]$p<0.05$ when compared to Negative control,[c]$p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison tests.

ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract

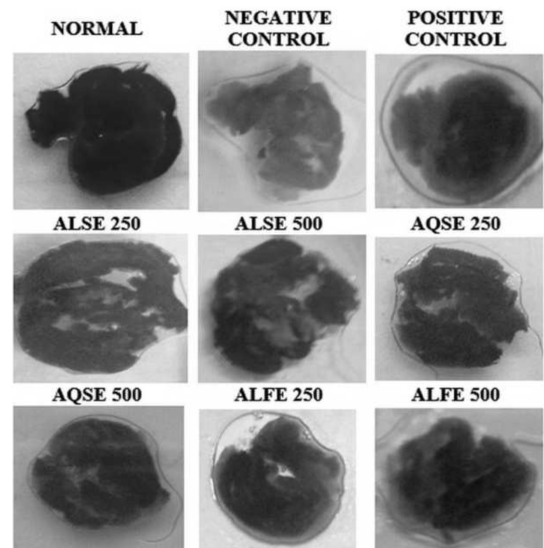

Fig. 5: Representative images of samples stained by Triphenyl tetrazolium chloride (TTC)

ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract

Biochemical analysis

Biochemical parameters in serum

The effect of various extracts on the serum parameters which includes CK-MB, LDH, Cholesterol, Triglycerides, SGOT and SGPT are shown in table 2 (a) and 2 (b). The activities of these enzymes were found to be increased significantly ($p<0.05$) in the negative control group when compared to the normal group. The treatment groups showed a significant decrease ($p<0.05$) in the enzyme levels when compared to the negative control group.

Table 2 (a): Effect of ALSE, AQSE and ALFE pre-treatment on cardiac marker enzymes in isoproterenol-induced myocardial infarction in rats

Groups	CKMB U/L	LDH U/L
Normal	11±0.26	101.25±0.83
Negative Control	31.75±0.59[a]	149.37±0.49[a]
Positive Control	14.12±0.71[b]	119.25±0.45[a,b]
ALSE 250	18.75±0.59[a,b,c]	132±0.65[a,b,c]
ALSE500	17.25±0.59[a,b,c]	125.75±0.79[a,b,c]
AQSE250	24.75±0.95[a,b,c]	136±0.84[a,b,c]
AQSE500	23.12±1.02[a,b,c]	131.75±0.64[a,b,c]
ALFE250	25.62±0.5[a,b,c] 9	134.5±0.62[a,b,c]
ALFE500	23.12±0.69[a,b,c]	132.25±0.64[a,b,c]

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at [a]$p<0.05$ when compared to Normal, [b]$p<0.05$ when compared to Negative control,[c]$p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison tests. ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract; CKMB-Creatine Kinase-Muscle/Brain Isoenzyme; LDH-Lactate Dehydrogenase

Table 2 (b): Effect of ALSE, AQSE and ALFE pre-treatment on cardiac marker enzymes in isoproterenol-induced myocardial infarction in rats

Groups	Cholesterol mg/dL	Triglycerides mg/dL	SGOT U/L	SGPT U/L
Normal	114±2.36	64.12±1.04	53.12±0.89	24.5±0.98
Negative Control	77.87±3.68^{a}	73.62±0.65^{a}	94.75±0.83^{a}	54.625±1.19
Positive Control	117.75±2.83^{b}	54.87±1.00^{a,b}	55.62±1.08^{b}	34.5±0.73^{a,b}
ALSE 250	66±0.80^{a,b,c}	66.12±0.83^{b,c}	63.5±0.86^{a,b,c}	50±1.53^{a,c}
ALSE500	62.87±0.71^{a,b,c}	63.75±0.59^{b,c}	58.87±1.41^{a,b,c}	42.5±0.82^{a,b,c}
AQSE250	66.37±0.86^{a,b,c}	67±0.92^{b,c}	67.12±0.71^{a,b,c}	50.375±0.88^{a,c}
AQSE500	65.75±0.92^{a,b,c}	64.37±0.84^{b,c}	63.12±0.78^{a,b,c}	45.125±1.02^{a,b,c}
ALFE250	63.62±0.53^{a,b,c}	65.37±1.10^{b,c}	73.87±0.91^{a,b,c}	46.625±1.29^{a,b,c}
ALFE500	61±0.70^{a,b,c}	62±0.84^{b,c}	67.12±0.98^{a,b,c}	52.875±1.66^{a,c}

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at $^{a}p<0.05$ when compared to Normal, $^{b}p<0.05$ when compared to Negative control,$^{c}p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison test. ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract; SGOT-Serum glutamic oxaloacetic transaminase; SGPT-Serum glutamic pyruvic transaminase

Table 3: Effect of ALSE, AQSE and ALFE pre-treatment on lipid peroxidation products and antioxidant systems in isoproterenol-induced myocardial infarction in rats

Groups	Lipid peroxidation MDA nmol/g protein	Superoxide dismutase U/mg	Catalase U/mg	Glutathione reductase U/mg
Normal	0.259±0.023	10.16±0.09	6.10±0.57	6.39±0.30
Negative Control	0.438±0.003^{a,b}	6.13±0.01^{a}	1.27±0.23^{a}	3.64±0.23^{a}
Positive Control	0.355±0.015^{a}	9.70±0.04^{b}	7.52±0.45^{b,c}	7.10±0.06^{b}
ALSE 250	0.403±0.004^{a,c}	9.21±0.31^{a,b}	4.41±0.34^{a,b,c}	5.95±0.03^{b,c}
ALSE500	0.370±0.004^{a,b}	9.84±0.16^{b}	4.82±0.31^{b,c}	5.60±0.27^{b,c}
AQSE250	0.383±0.000^{a,b}	7.75±0.14^{a,b,c}	3.93±0.19^{a,b,c}	7.94±0.21^{a,b}
AQSE500	0.322±0.004^{a,b}	7.14±0.17^{a,b,c}	4.46±0.21^{a,b,c}	6.53±0.22^{b}
ALFE250	0.356±0.003^{a,b}	6.24±0.21^{a,c}	3.48±0.21^{a,b,c}	9.09±0.24^{a,b,c}
ALFE500	0.323±0.003^{a,b}	6.99±0.05^{a,b,c}	4.68±0.30	8.83±0.32^{a,b,c}

Results are expressed as mean±SEM, for eight rats in each group. Statistical significance at $^{a}p<0.05$ when compared to Normal, $^{b}p<0.05$ when compared to Negative control,$^{c}p<0.05$when compared to Positive control based on one-way analysis of variance followed by Tukey's multiple comparison test. ALSE-Alcoholic Seed Extract; AQSE-Aqueous Seed Extract; ALFE-Alcoholic Fruit Extract; MDA-Malondialdehyde Equivalent

Assay of lipid peroxidation products and antioxidant systems

The levels of lipid peroxidation, superoxide dismutase, catalase and glutathione reductase are shown in table 3. Their levels were significantly decreased ($p<0.05$) in the heart tissue homogenate of negative control group when compared to the normal group, while an increased level of lipid peroxidation was seen in the negative control group when compared with normal group. Pre and co treatment with the extracts showed a significant increase in the levels of superoxide dismutase, catalase, and glutathione reductase when compared to negative control and a decrease in lipid peroxidation enzyme level was observed in treatment groups when compared to negative control group

DISCUSSION

Results of the present investigation clearly revealed the cardioprotective effects of ALSE, AQSE and ALFE in rat model of experimentally induced myocardial necrosis. While IPRT administration caused myocardial injury, pre, and co-treatment with ALSE, AQSE and ALFE could protect the heart from IPRT induced cardiac damage.

It's established that catecholamines regulate the metabolism and contractility of the myocardium, and in large doses accounts for cellular damage, observed in clinical conditions like angina, transient myocardial hypoxia, acute coronary insufficiency and subendocardial infarct, the alterations shown in biochemical parameters like increased lipid peroxidation besides an elevated level of myocardial lipids as well as altered activities of the cardiac enzymes and antioxidants are evidence of the same [6–8,25,26]. One of the various mechanisms proposed to elucidate the effect of IPRT a synthetic catecholamine and a β-adrenergic agonist to explain the IPRT induced cardiotoxicity is by the generation of highly cytotoxic free radicals through auto-oxidation of catecholamines which has been implicated as one of the important causative factors [27].

Following IPRT administration, the alterations in the electro-cardiogram, lipid peroxidation, antioxidants endogenous enzymes; activities of cardiac markers such as LDH and CK-MB and cytosolic enzymes such as cholesterol, triglycerides, SGOT and SGPT were aggravated [28]. Further results of the TTC assay are indicating the extent of myocardial necrosis also confirmed the cardiotoxic effects of IPRT. But IPRT induced animals which were pre or co-treated with the ALSE, AQSE and ALFE; all these deleterious effects were minimized suggesting the amelioration of cardiac abnormalities.

The primary purpose of ECG is to detect infarction or acute coronary injury. The observed ST segment elevation in isoproterenol induced myocardial infarcted rats might be due to myocardial necrosis caused by isoproterenol. This is supported by a study stating that acute ischemic tissue injury manifested ST segment elevation in the region of the injured myocardium [29]. Pre or co-treatment with ALSE, AQSE and ALFE in isoproterenol induced myocardial infarcted rats showed normal ECG, suggestive of its cell membrane protective effects. A significant increase in the heart weight, with a significant change in body weight, was observed which might be attributed to increased water content, oedematous intramuscular space [30] and increased protein content. Pre or co-treatment with ALSE, AQSE, and ALFE bought down the heart weight which an indicative of its myocardium protection against infiltration and it also could be due to the decrease in water content of the myocardium.

Staining of cardiac tissue slices with TTC is an accepted method to assess necrosis of myocardial tissue [31]. Therefore, very often extent of myocardial infarction is detected by direct staining with TTC dye, which forms a red formazan precipitate in the presence of intact dehydrogenase enzyme. Whereas the infarcted myocardium lacks dehydrogenase activity and therefore fails to stain with it. Area of infarction may relate to leakage of dehydrogenases and loss of membrane integrity [32]. IPRT-induced rats showed a significant increase in necrotic area with less TTC absorbing capacity, indicating significant leakage of dehydrogenases from the myocardium. On the other hand, ALSE, AQSE and ALFE pre or co-treated IPRT induced rats depicted the minimal area of necrosis, indicating only a mild leakage of LDH, further showing a better protection from cardiac damage.

Lipid peroxidation is a well-established mechanism of cellular injury and has been used as an indicator of oxidative stress [33, 34]. Increased levels of thiobarbituric acid reactive substances in cardiac tissue indicate excessive production of free radicals and decreased antioxidant systems in myocardial infarcted rats. Pre-co-treatment with ALSE, AQSE and ALFE decreased the levels of lipid peroxidation products in the heart of isoproterenol-induced myocardial infarcted rats. Thus, ALSE, AQSE and ALFE scavenge excessive free radicals produced by IPRT in myocardial infarcted rats and protect the myocardium.

The decline in the activities of superoxide dismutase and catalase in isoproterenol-induced cardiac tissue might be due to superoxide radicals generated at the site of damage, which modulates superoxide dismutase and catalase resulting in decreased activities which lead to the accumulation of superoxide anion and in consequence damage myocardium [35]. Pre-co-treatment with ALSE, AQSE and ALFE improved the activities of these enzymes in isoproterenol-induced myocardial infarcted rats. Thus, ALSE, AQSE and ALFE scavenge superoxide radicals and reduce myocardial damage caused by free radicals in cardiac tissue in isoproterenol induced myocardial infarcted rats.

The decreased activity of cardiac tissue glutathione reductase is due to decreased concentration of its substrate reduced glutathione in isoproterenol-induced rats [35]. The enhanced protective mechanism towards oxidative stress in myocardial infarction may consume reduced glutathione and depress reduced glutathione levels [36, 37]. Prior treatment with ALSE, AQSE and ALFE improved the levels of reduced glutathione in isoproterenol-induced myocardial infarcted rats. Increased levels of reduced glutathione reveal only that there is less radical formation and consequently less formation of oxidized glutathione. The enhanced concentration of reduced glutathione observed in ALSE, AQSE and ALFE pre-co-treated isoproterenol-induced myocardial infarcted rats resulted in enhanced activity of glutathione reductase and prevented cellular damage in cardiac tissue.

The underlying mechanism of action of ALSE, AQSE and ALFE *in vitro* was also studied. In this study, free radical scavenging activity of ALSE, AQSE and ALFE was determined by DPPH, ABTS and superoxide scavenging methods. It has been shown that free radical DPPH, ABTS and Superoxide anion scavenging is widely used to evaluate the free radical scavenging effects of various antioxidant substances [38]. ALSE, AQSE and ALFE scavenges free radical DPPH, ABTS and Superoxide anion dose dependently. Increased free radical production is one of the biochemical mechanisms in isoproterenol-induced myocardial infarction. Isoproterenol metabolism produces increased free radicals such as superoxide anions and hydroxyl radicals. ALSE, AQSE and ALFE protect the myocardium by scavenging these excessive free radicals produced by isoproterenol due to its free radical scavenging effect. The *in vitro* study supports the free radical scavenging effects of ALSE, AQSE and ALFE.

CONCLUSION

In conclusion, the present study demonstrated that subcutaneous injections of IPRT produced myocardial infarction in rats which is evident by the release of myocyte injury markers in serum as well as other cytosolic enzymes. Myocardial lesions were associated with decreased antioxidant defence status, heart electrocardiographic changes and release of inflammatory markers. In addition, the present study provided experimental evidence that ALSE, AQSE and ALFE maintained the antioxidant enzyme levels and improved cardiac performance following high-dose isoproterenol administration. This finding might be a scientific support to understand the beneficial effects of ALSE, AQSE and ALFE on cardioprotection against myocardial injury, in which oxidative stress has long been known to contribute to the pathogenesis and it can be reproduced in human beings, these findings may present a novel prophylactic therapy for myocardial infarction.

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CONFLICT OF INTERESTS

We declare that we have no conflicts of interest

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