BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Attenuation of Infarct Size by Leucas aspera in Ischemia-Reperfusion Induced Myocardial Infarction in Rats

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ABSTRACT

The chronic administration of aqueous and alcoholic extracts of *Leucas aspera* (*L. aspera*) was evaluated for protection against ischemia–reperfusion (I/R) induced myocardial infarction in rats. Aqueous and alcoholic extracts of *L. aspera* were administered orally to wistar albino rats (200 – 250 g) in three different doses (100, 250 and 500 mg kg^{-1}), by gastric gavage for one week. At the end of this period, control (I/R) group and experimental groups were subjected to 30 min occlusion of the left anterior descending coronary artery and thereafter reperfused for 4 h. Infarct size was measured by using the staining agent 2,3,5-triphenyl tetrazolium chloride (TTC). Ischemia – reperfusion induced cardiac necrosis was evidenced by elevated levels of the serum marker enzymes such as SGOT, SGPT LDH, CK-MB and CK and lipid peroxides in serum and heart tissue, increased levels of myeloperoxidase (MPO) in heart tissue. Oxidative damage was further evidenced by decreased levels of antioxidative enzymes SOD, CAT, GST and GSH in the hearts subjected to *in vivo* myocardial ischemia - reperfusion injury. The pretreatment with *L. aspera* produced a significant reduction in infarct size and decreased levels of lipid peroxides in serum and heart tissue. The plant extract dose dependently inhibited the rise in serum marker enzymes and antagonized the reduction in antioxidant enzymes in heart tissue. The present study clearly indicates that *L. aspera* by neutralizing the cytotoxic free radicals generated during I/R injury, thereby protects the loss of membrane integrity and stabilizing the membrane, hence protects the myocyte from oxidative stress. This is further conformed by the reduced levels of myeloperoxidase indicating that pretreatment with aqueous and alcoholic extracts suppressed neutrophil infiltration into the injured myocardium and protects rat heart against ischemia-reperfusion induced myocardial infarction.

Keywords: Ischemia – reperfusion, Free radicals, Neutrophil infiltration.

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Received 29 February 2016, Accepted 03 March 2016

Please cite this article as: Atluri LD *et al*., Attenuation of Infarct Size by Leucas aspera in Ischemia-Reperfusion Induced Myocardial Infarction in Rats . British Journal of Medical and Health Research 2016.

INTRODUCTION

Free radical attacks are involved in the pathophysiology of a wide variety of disease conditions including ischemic heart disease, resulting from different endogenous antioxidant defenses [1]. One of the major causes of ischemic heart disease and heart failure is an imbalance between oxidants and antioxidant defenses. Thus it may be possible to limit oxidative stress induced tissue damage and hence, prevent or ameliorate disease progression favoring the balance towards lower oxidative stress. There is comprehensive experimental and clinical evidence that either exogenous supplementation of natural antioxidants or augmentation of endogenous antioxidants such as superoxide dismutase, catalase, reduced glutathione and glutathione S-transferase attenuates the myocardial infarction [2,3]. Indeed, many antioxidative plants and their isolated components have been reported to possess cardioprotection in experimental models of myocardial ischemia reperfusion injury [4-16]. Our previous studies also suggested the cardioprotective activity of *Hydrocotyle asiatica* and *Tinospora cordifolia* against ischemia reperfusion injury[17, 18].

Leucas aspera (*L. aspera*) belonging to the family Labiatae, is commonly called as "Chota halkusa". It grows as a weed on wastelands and roadsides all over India. It is also native of Africa, Mauritius, South Asia and tropical zones. Entire plant is used as an insecticide and indicated in traditional medicine for cough, cold, painful swellings and chronic skin eruptions [19]. The leaves and flowers are useful in colic, dyspepsia, antipyretic, chronic rheumatism, arthralgia, psoriasis, ulcers, anthelmintic, and antibacterial [19]. The juice of leaves is highly recommendable as an eye drop in encephalopathy due to warm infestation in children. Compounds isolated from the plant include long-chain aliphatic compounds, triterpenes, sterols-sitosterol, stigmasterol, campesterol and a novel phenolic compound [20-23]. *L. aspera* has been reported to possess a variety of biological activities, including anti-inflammatory, analgesic [24-26], antimicrobial [27], prostaglandin inhibitory [28] and antioxidant activities, etc. These biological activities, in some terms, may be partially related to antioxidant activity [28].

In the present study we investigated the cardioprotective activity of aqueous and alcoholic extracts of *L. aspera* against ischemia-reperfusion induced myocardial injury. The dose (100, 250 and 500 mg/kg) dependent protection of aqueous and alcoholic extracts were studied by investigating the infarct size limiting effect, lipid peroxide levels in serum and heart tissue, endogenous antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and reduced glutathione (GSH)) and serum marker enzymes (serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), creatine kinase–isoenzyme (CK-MB) and total

creatine (CK)). Myeloperoxidase activity was studied against 500 mg/kg dose of both aqueous and alcoholic extracts.

MATERIALS AND METHOD

Chemicals

2,3,5- triphenyl tetrazolium chloride (TTC) was purchased from BDH chemicals Ltd (England) 1,1,3,3-tetraethoxypropane was purchased from Sigma Chemical Company (St. Louis, USA). Thiopentone sodium was supplied by Abott Lab Ltd. (Ankleshwar, India) Nitroblue tetrazolium (NBT), NADH, Reduced glutathione, oxidized glutathione, 1-chloro 2,4-dinitro benzene, Folin-phenol reagent, Ellman' s reagent, TritonX-100 RS were purchased from Sisco Research Laboratories Pvt Ltd., (Mumbai, India). Phenazine methosulphate was purchased from National Chemicals (Vadodara, India). All other chemicals and reagents used were of analytical grade.

Animals

Albino Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200 – 250 g were selected. Animals were maintained under standard laboratory conditions at 25 ± 2^0 C relative humidity 50 ± 15% and normal photoperiod (12 h dark/ 12 h light and were used for the experiment commercial pellet diet (Ratan Brothers, India) and water were provided *ad libitum.* The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government (Regd. No. 516/01/A/CPCSEA).

Plant material

The whole plant of *Leucas aspera* was collected from Coastal Districts of Andhra Pradesh, South India. Dr. K Hemadri, taxonomist, Regional Research Institute, Botanical Survey of India, Vijayawada, identified the herb. The specimens were preserved in the herbarium of our department.

Preparation of extract

For the preparation of alcoholic extract, shade dried powdered (750 g) of *L. aspera* was extracted with alcohol (95% v/v) by Soxhlation until there is no compound left over in solvent. The crude extract was evaporated to dryness in a rotary film evaporator (15.5 % w/w). For the preparation of aqueous extract, coarse powdered of plant was extracted with eight parts of water and was concentrated by boiling for 5 h at reduced temperature and pressure in rotary evaporator. The filtrate was used as aqueous extract for the experiment.

Experimental design

The rats were divided into six groups, each consisting of five animals. Group I treated with 1% Sodium CMC orally and served as sham control; Group 2 treated with 1% Sodium CMC

orally and served as a control I/R, Group 3, 4 and 5 were treated with aqueous extract of *L. aspera* at doses 100, 250 and 500 mg kg^{-1} respectively. Group 6, 7 and 8 were treated with alcoholic extract of *L. aspera* at doses 100, 250 and 500 mg kg^{-1} respectively. Group 9 was treated with ramipril at a dose of 2 mg kg^{-1} served as reference standard. On day 8^{th}, 1 h after the above treatments, rats were subjected to 30 min LAD coronary artery occlusion and followed by 4 h reperfusion. Sham control group was not subjected to 30 min LAD coronary artery occlusion.

Surgical procedure

Coronary artery occlusion and reperfusion was performed according to the method described by Pragada et al [17]. In brief, rats were anaesthetized with thiopentone sodium (30 mg kg^{-1}, i.p.). The neck was opened with a ventral midline incision and intubated through a tracheotomy and ventilated with room air by a techno positive pressure respirator (Crompton Parkinson Ltd., England). The body temperature was monitored and maintained at $37^{O}C$ through out the experimental protocol. A left thorocotomy and pericardiotomy were performed, followed by identifying the marginal branch of the left anterior descending coronary artery (LAD). A silk thread (4-0) was passed behind the artery and was occluded by a knot for 30 min. The silk thread was removed after 30 min with the help of two knot releasers to allow reperfusion of the heart for succeeding 4 h.

Quantification of infarct size

In all the groups after sacrificing the animal by injecting 2.56 M potassium chloride directly into the left ventricle, the heart was excised from the thorax rapidly and the greater vessels were removed. The left ventricle was separated from the heart and was weighed. It was sliced parallel to the atrioventricular grove into 2-3 mm thick sections and the slices were incubated in 1% TTC solution prepared in phosphate buffer pH 7.4 for 30 min at $37^{O}C$ [29] In viable myocardium TTC is converted by dehydrogenases to a red formazan pigment that stains tissue dark red [30]. The infarcted myocardium that does not take TTC stain where the dehydrogenases are drained off, remains pale in color, the pale necrotic tissue was separated from the stained portions and weighed on a electronic balance (Dhona 200D). Infarct size was calculated as a percentage fraction of non-viable myocardium of the left ventricle.

Biochemical Studies

In all the groups before sacrificing the animal, blood samples were collected and the maker enzymes such as SGOT, SGPT, LDH, CK-MB and CK in serum were estimated spectrophotometrically using kits from Reckon Diagnostics Pvt Ltd., Gorwa Baroda, India, and Randox Laboratories Ltd., U.K. respectively. Lipid peroxide levels in terms of MDA in serum and heart tissue were estimated by the method developed by Yagi and Ohkawa et al

respectively [31, 32] and were expressed as nmol ml^{-1} and nmol of g^{-1} tissue in serum and heart tissue respectively. Protein was estimated by the method of Lowry et al [33] and expressed as mg protein. g^{-1} tissue. Reduced glutathione was estimated by the method of Ellman [34] and expressed as micrograms/ g wet tissue. Glutathione S-transferase was assayed by the method of Habig et al [35] and expressed as nmoles of CDNB conjugated/mg protein/min. Superoxide Dismutase was estimated by the method described by Kakkar et al. [36] and expressed as Units mg $protein^{-1}$. Catalase levels were measured by the method of Aebi [37] and expressed as micromoles of H_2O_2 metabolized/ mg protein/min.

Myeloperoxidase assay

Myeloperoxidase, a green hemoprotein enzyme stored in the granules of neutrophils can utilize hydrogenperoxide generated by NADPH oxidase to oxidize halides (cl^-, Br^-, I^- to their corresponding hypohalous acids an additional class of active oxygen metabolites). Myeloperoxidase (MPO) activity was used as a marker for neutrophil content in the heart, since it correlates closely with the number of neutrophils [38]. In order to quantify myocardial neutrophil infiltration, the cardiac activity of myeloperoxidase, a plentiful enzyme of neutriophils, was assessed using the method modified from that of Mullane et al [39.] The myocardial tissue was homogenized in 50mM K_2HPO_4 buffer (pH 6) containing 0.5% hexadecyl trimethylammonium bromide (HTBA) using a Polytron (Ultra-turrax T-25) homogenizer. After freeze-thawing for three times, the samples were centrifuged at 15000 rpm for 30 min at 4^0C and the resulting supernatant assayed spectrophotometrically for MPO determination. In brief, 40 µl of sample was mixed with 960 µl of 50 mM phosphate buffer pH 6, containing 0.167 mg/ml *O*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured with Spectrophotometer. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity data are presented as units per g tissue.

Statistical analysis

The results are expressed as means ± S.E.M., the data was processed by factorial One-way analysis of Variance. Individual groups were compared using Dunnet's *t* test. Difference with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect of L. aspera on myocardial infarct size

In sham control and control groups (Group 1 and 2), the infarct size was found to be 4.50 ± 0.79 and 50.9 ± 1.90 respectively and the infarct size in control group was statistically significant compared to sham control group. In groups treated with aqueous extract of *L.*

aspera (Group 3, 4 and 5) the infarct size was decreased to 39.93 ± 0.42, 26.79 ± 1.00 and 15.49 ± 0.37 respectively. In groups treated with alcoholic extract of *L. aspera* (Groups- 6, 7 and 8) the infarct size was decreased to 40.86 ± 0.29, 35.18 ± 0.79 and 18.19 ± 0.24 respectively and in ramipril treated group the infarct size was decreased to 17.6 ± 0.8 and the difference was statistically significant ($P < 0.05$) compared to control (Table 1). Lipid peroxide levels in serum and heart tissue of sham control group were found to be 3.70 ± 0.44 nmol ml^{-1} and 8.20 ± 0.25 nmol g^{-1} tissue respectively. Lipid peroxide levels in serum and heart tissue of control group were found to be 27.6 ± 1.42 nmol ml^{-1} and 109.2 ± 2.77 nmol g^{-1} tissue respectively and statistically significant compared to sham control group. In Group- 3, 4 and 5 the lipid peroxide levels in serum were found to be 21.73 ± 0.53, 16.79 ± 0.52, 15.86 ± 0.55 nmol ml^{-1} and in heart tissue the lipid peroxide levels were found to be 67.26 ± 1.24, 34.79 ± 0.54 and 25.56 ± 0.37 nmol g^{-1} tissue respectively. In Group- 6, 7 and 8 the lipid peroxide levels in serum were found to be 19.54 ± 0.64, 18.19 ± 1.14, and 16.81 ± 1.14 nmol ml^{-1} and in heart tissue were found to be 43.10 ± 2.28, 27.07 ± 0.38 and 18.85 ± 0.44 nmol g^{-1} tissue respectively. In Group-9, ramipril significantly decreased the lipid peroxide levels in serum and heart tissue and were found to be 4.3 ± 0.4 nmol ml^{-1} and 30.8 ± 1.9 nmol g^{-1} tissue respectively. The results are given in table 1.

Effect on myocardial antioxidants

In sham control and control groups, the SOD levels in heart tissue were found to be 25.24 ± 1.21 and 13.76 ± 0.57 Units/mg protein respectively and the levels in control group were statistically significant compared to sham control group. The SOD levels in heart tissue of groups treated with aqueous extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 13.35 ± 0.22, 17.35 ± 0.29 and 19.85 ± 0.19 Units/mg protein respectively. The SOD levels in heart tissue of groups treated with alcoholic extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 12.78 ± 0.48, 17.26 ± 0.22 and 19.27 ± 0.24 Units/mg protein respectively.

In sham control and control groups, the CAT levels in heart tissue were found to be 26.99 ± 0.18 and 12.42 ± 0.578 micromoles of H_2O_2 metabolized/ mg protein/min respectively and the levels in control group were statistically significant compared to sham control animals. The CAT levels in heart tissue of groups treated with aqueous extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 12.93 ± 0.37, 17.47 ± 0.24 and 19.34 ± 0.38 micromoles of H_2O_2 metabolized/ mg protein/min respectively. The CAT levels in heart tissue of groups treated with alcoholic extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were

found to be 12.34 ± 0.39, 16.69 ± 0.33 and 18.68 ± 0.21 micromoles of H_2O_2 metabolized/ mg protein/min respectively.

In sham control and control groups, the GST levels in heart tissue were found to be 0.92 ± 0.012 and 0.494 ± 0.026 nmoles of CDNB conjugated/mg protein/min respectively and the levels in control group were statistically significant compared to sham control group. The GST levels in heart tissue of groups treated with aqueous extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 0.55 ± 0.01, 0.68 ± 0.019 and 0.79 ± 0.014 nmoles of CDNB conjugated/mg protein/min respectively. The GST levels in heart tissue of groups treated with alcoholic extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 0.53 ± 0.01, 0.63 ± 0.015 and 0.78 ± 0.022 nmoles of CDNB conjugated/mg protein/min respectively.

In sham control and control groups, the GSH levels in heart tissue were found to be 442.38 ± 14.87 and 80.42 ± 9.163 micrograms/g wet tissue respectively and the levels in control group were statistically significant compared to sham control group. The GSH levels in heart tissue of groups treated with aqueous extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 104.09 ± 17.18, 187.04 ± 12.59 and 345.7 ± 12.58 micrograms/g wet tissue respectively. The GSH levels in heart tissue of groups treated with alcoholic extract of *L. aspera* at dose of 100, 250 and 500 mg kg^{-1} were found to be 70.65 ± 6.93, 130.11 ± 15.52 and 249.06 ± 13.12 micrograms/g wet tissue respectively and the difference was also significant ($P< 0.05$) compared to control. The results are given in table 2.

Table 1: Effect of aqueous and alcoholic extracts of *Leucas aspera* on infarct size and lipid peroxide levels in serum and heart tissue of control and experimental group of rats

Treatment	Dose (mg/kg)	Infarct size (% Left Ventricle Necrosis)	Lipid peroxide levels in serum(nmol/ml)	Lipid peroxide levels in Heart Tissue (nmol/gm wet tissue)
Sham control	-----	4.50 ± 0.79 [a]	3.70 ± 0.44 [a]	8.20 ± 0.25 [a]
Control	------	50.91 ± 1.90	27.57 ± 1.42	109.24 ± 2.77
Aqueous *L aspera*	100	39.93 ± 0.42 [a]	21.73±0.53 [a]	67.26 ± 1.24 [a]
Aqueous *L aspera*	250	26.79 ± 1.00 [a,b]	16.79±0.52 [a,b]	34.79 ± 0.54 [a,b]
Aqueous *L aspera*	500	15.49 ± 0.37 [a,b,c]	15.86 ± 0.55 [a,b]	25.56 ± 0.37 [a,b,c]
Alcoholic *L aspera*	100	40.86 ± 0.29 [a]	19.54 ± 0.64 [a]	43.10±2.28 [a]
Alcoholic *L aspera*	250	35.18 ± 0.79 [a,d]	18.19 ± 1.14 [a,]	27.07 ± 0.38 [a,d]
Alcoholic *L aspera*	500	18.19 ± 0.24 [a,d,e]	16.81 ±1.14 [a,#]	18.85 ± 0.44 [a,d,e]
Ramipril	2	17.60 ± 0.80 [a]	4.3 ± 0.40 [a]	30.80 ± 1.9 [a]

All values are expressed as Mean ± S.E.M. (n =5). [a]p<0.001, as compared to control, [b]p<0.001, as compared to aqueous 100 mg/kg; [c]p<0.001, as compared to aqueous 250 mg/kg; [d]p<0.001, as compared to alcoholic 100 mg/kg; [e]p<0.001, as compared to alcoholic 250 mg/kg. [#]p<0.05, as compared to alcoholic 100 mg/kg.

Table 2: Effect of aqueous and alcoholic extracts of *Leucas aspera* on antioxidant enzymes in the heart of control and experimental group of rats

Treatment	Dose (mg/kg)	Superoxide Dismutase (U/mg protein)	Catalase(μ moles of H_2O_2 metabolized /mg protein/min)	Reduced Glutathione (μg/g wet tissue)	Glutathine-S-trasferase (nmoles of CDNB conjugated /mg protein/min)
Sham control	-----	25.24 ± 1.216 [a]	26.99 ± 0.181 [a]	442.38 ± 14.87 [a]	0.92 ± 0.012 [a]
Control	------	13.76 ± 0.578	12.42 ± 0.578	80.42 ± 9.163	0.49 ± 0.026
Aqueous *L aspera*	100	13.35 ± 0.223	12.93 ± 0.379	104.09 ± 17.18	0.55 ± 0.016
Aqueous *L aspera*	250	17.35 ± 0.291 [a,b]	17.47 ± 0.245 [a,b]	187.04 ± 12.59 [a,b]	0.68± 0.019 [a,b]
Aqueous *L aspera*	500	19.85 ± 0.199 [a,b,c]	19.34 ± 0.383 [a,b,c]	345.7 ± 12.58 [a,b,c]	0.79 ± 0.014 [a,b,c]
Alcoholic *L aspera*	100	12.78 ± 0.484	12.34 ± 0.398	70.65 ± 6.933	0.53 ± 0.012
Alcoholic *L aspera*	250	17.26±0.224 [a,d]	16.69±0.338 [a,d]	130.11±15.526 [#,d]	0.63±0.015 [a,d]
Alcoholic *L aspera*	500	19.27 ± 0.242 [a,d,e]	18.68 ± 0.216 [a,d,e]	249.06 ± 13.121 [a,d,e]	0.75 ± 0.022 [a,d,e]
Ramipril	2	21.67 ± 0.505 [a]	12.392 ± 0.28 [a]	401.46 ± 11.165 [a]	0.74 ± 0.01 [a]

Effect on serum marker enzymes

In sham control group, the marker enzymes such as SGOT, SGPT, LDH, CK-MB and CK, levels in serum were found to be 179.3 ± 4.3, 55 ± 3.79, 510 ± 9.3, 146 ± 4.16 and 506.3 ± 17.64 IU/L respectively. In control group, the serum marker enzyme levels were found to be 1324 ± 6.64, 435.5 ± 3.28, 6037 ± 6.3, 4378.66 ± 61.23 and 5551 ± 95.19 IU/L respectively and the levels were statistically significant compared to sham control group. The serum marker enzymes levels in groups treated with aqueous extract and alcoholic extract of *L. aspera* at dose of 100 mg kg^{-1} were found to be 648 ± 3.21, 204.66 ± 35.5, 2041.33 ± 47.19, 909 ± 24.13, 3019 ± 26.75 and 687 ± 6.35, 206.6 ± 6.016, 2523 ± 34.8, 1149.33 ± 49.8, 3368.3 ± 28.14 IU/L respectively. At a dose of 250 mg kg^{-1}, the serum marker enzymes levels in groups treated with aqueous extract and alcoholic extract of *L. aspera* were found to be 465.66 ± 7.31, 152.33 ± 2.4, 1270 ± 12.74, 710.33 ± 19.81, 2361 ± 28.14 and 420.33 ± 5.2, 175.66 ± 3.84, 1258 ± 38.2, 894 ± 5.85, 2782 ± 35.17 IU/L respectively. At a dose of 500 mg kg^{-1}, the serum marker enzymes levels in groups treated with aqueous extract and alcoholic extract of *L. aspera* were found to be 235.66 ± 3.84, 80.66 ± 4.26, 706 ± 24.3, 339.66 ± 5.69, 1240 ± 3.2 and 262.33 ± 4.93, 101 ± 1.33, 997.33 ± 6.65, 455.66 ± 20.8, 1317.3 ± 11.6 IU/L respectively and the difference was also significant (P< 0.05) compared to control. The results are shown in figure 1-5.

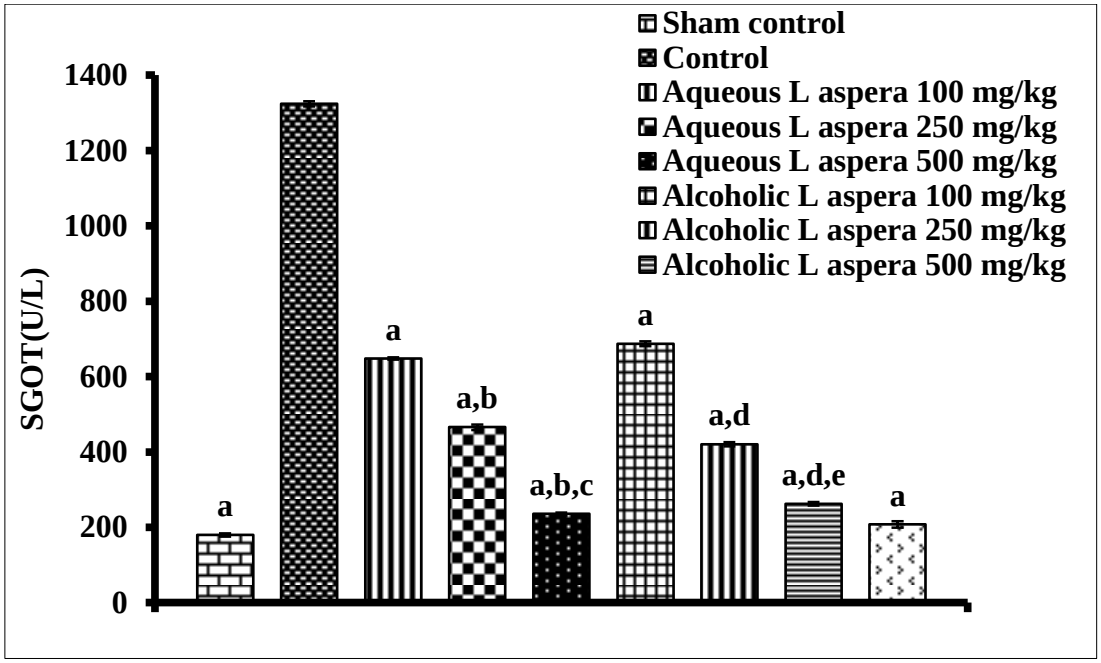

Figure 1*:* Effect of aqueous and alcoholic extracts of *Leucas aspera* on SGOT levels of experimental group animals

All values are expressed as Mean ± S.E.M. (n =5). [a]p<0.001, as compared to control, [b]p<0.001, as compared to aqueous 100 mg/kg; [c]p<0.001, as compared to aqueous 250 mg/kg;

[d]p<0.001, as compared to alcoholic 100 mg/kg; [e]p<0.001, as compared to alcoholic 250 mg/kg.

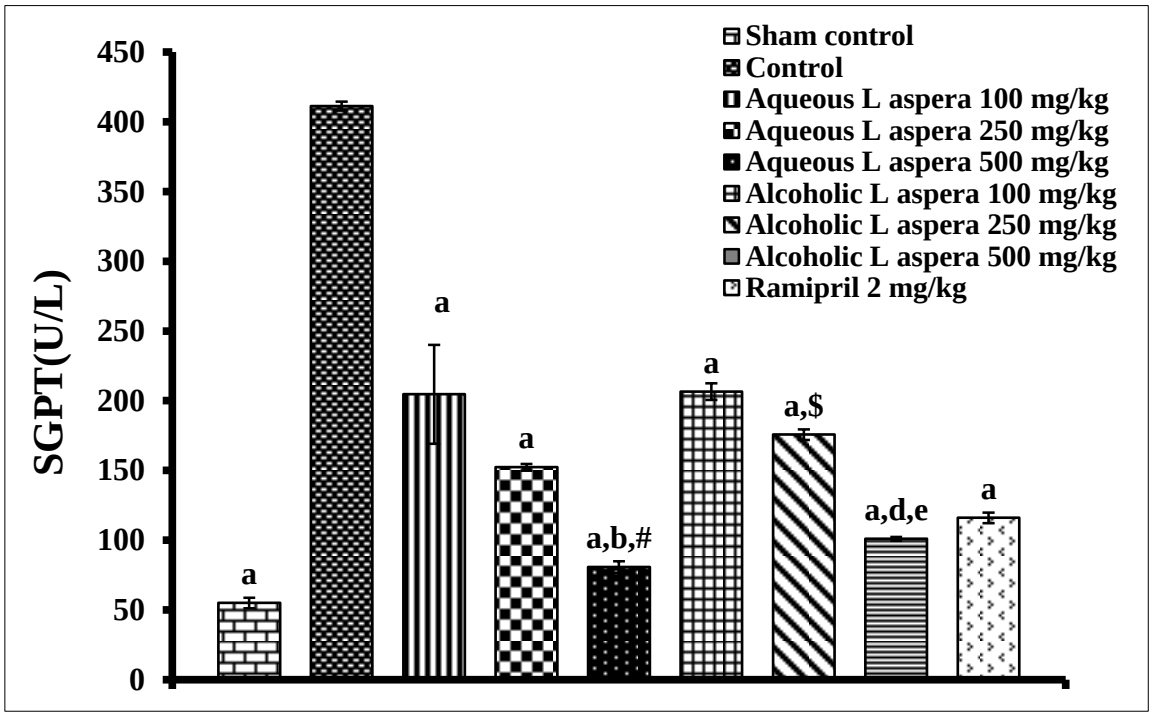

Figure 2: Effect of aqueous and alcoholic extracts of Leucas aspera on SGPT levels of experimental group animals

All values are expressed as Mean ± S.E.M. (n =5). [a]p<0.001, as compared to control, [b]p<0.001, as compared to aqueous 100 mg/kg; [#]p<0.05, as compared to aqueous 100 mg/kgl; [d]p<0.001, as compared to alcoholic 100 mg/kg; [e]p<0.001, as compared to alcoholic 250 mg/kg. [$]p<0.01, as compared to alcoholic 100 mg/kg.

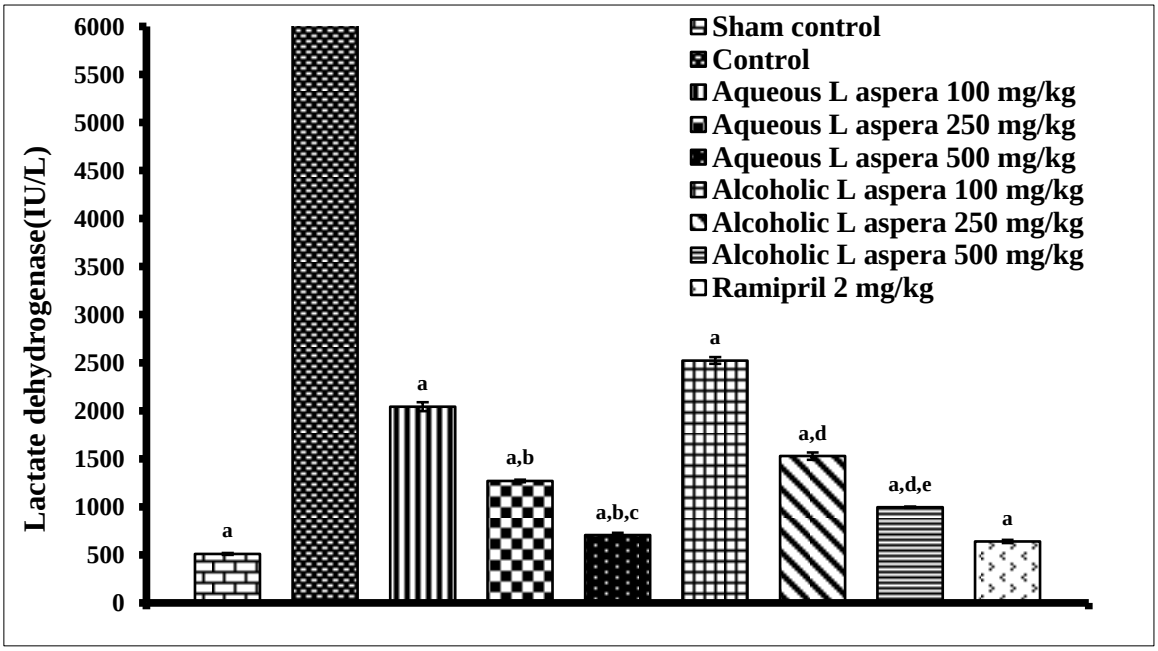

Figure 3: Effect of aqueous and alcoholic extracts of Leucas aspera on LDH levels of experimental group animals

All values are expressed as Mean ± S.E.M. (n =5). [a]p<0.001, as compared to control, [b]p<0.001, as compared to aqueous 100 mg/kg; [c]p<0.001, as compared to aqueous 250 mg/kg; [d]p<0.001, as compared to alcoholic 100 mg/kg; [e]p<0.001, as compared to alcoholic 250 mg/kg.

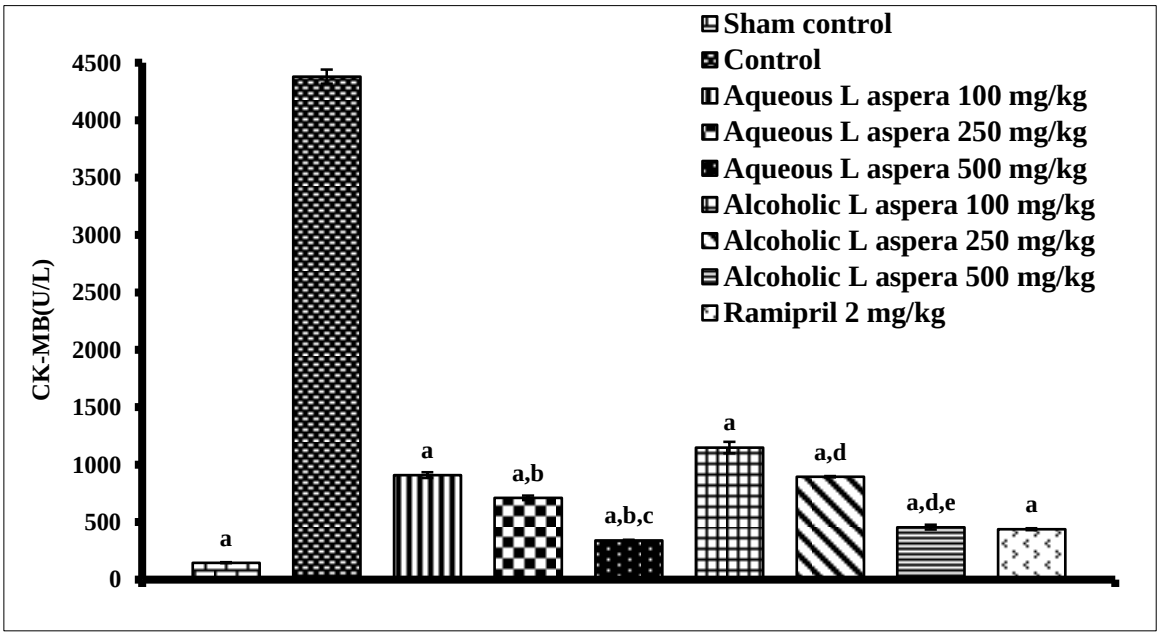

Figure 4: Effect of aqueous and alcoholic extracts of Leucas aspera on CK-MB levels of experimental group animals

All values are expressed as Mean ± S.E.M. (n =5). [a]p<0.001, as compared to control, [b]p<0.001, as compared to aqueous 100 mg/kg; [c]p<0.001, as compared to aqueous 250 mg/kg; [d]p<0.001, as compared to alcoholic 100 mg/kg; [e]p<0.001, as compared to alcoholic 250 mg/kg.

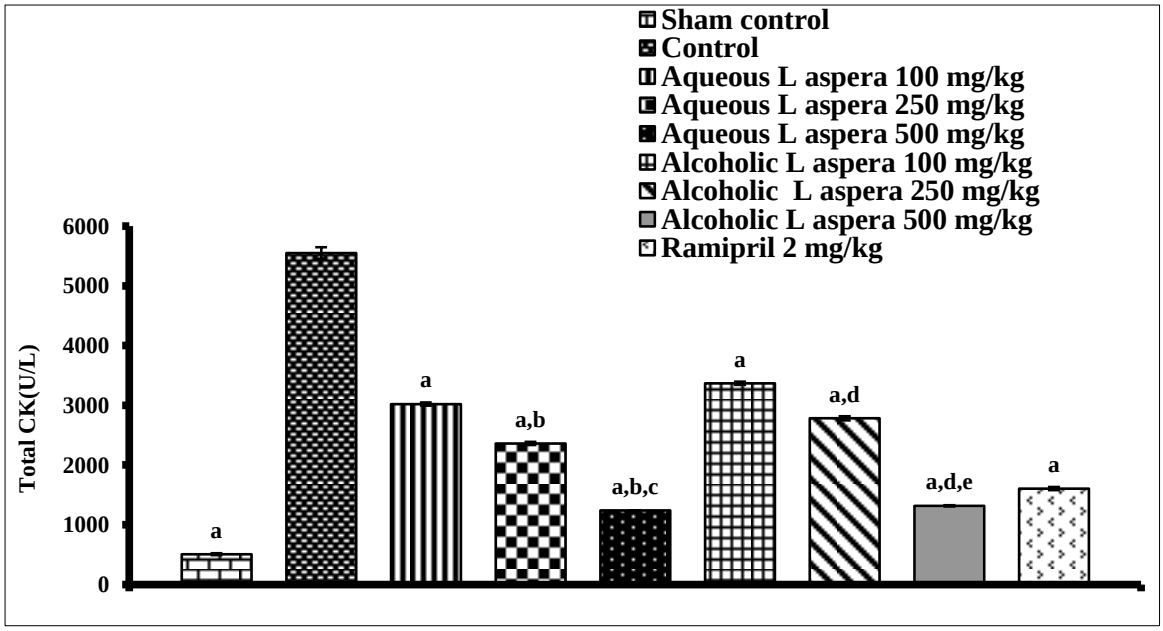

Figure 5: Effect of aqueous and alcoholic extracts of *Leucas aspera* on total CK levels of experimental group animals

All values are expressed as Mean ± S.E.M. (n =5). [a]p<0.001, as compared to control, [b]p<0.001, as compared to aqueous 100 mg/kg; [c]p<0.001, as compared to aqueous 250 mg/kg; [d]p<0.001, as compared to alcoholic 100 mg/kg; [e]p<0.001, as compared to alcoholic 250 mg/kg.

Effect on myeloperoxidase levels

In control group, the myeloperoxidase level in heart tissue was found to be 11.68 ± 0.18 U/ g wet tissue and statistically significant compared to sham control group (2.4 ± 0.14 U/ g wet tissue). Pretreatment with aqueous and alcoholic extracts of *L. aspera* at a dose of 500 mg kg^{-1} the myeloperoxidase level were found to be 4.04 ± 0.27 and 3.88 ± 0.34 U/ g wet tissue. In ramipril (2 g kg^{-1}) treated group, myeloperoxidase level was found to be 5.96 ± 0.33 U/ g wet tissue. The results were shown in figure 6.

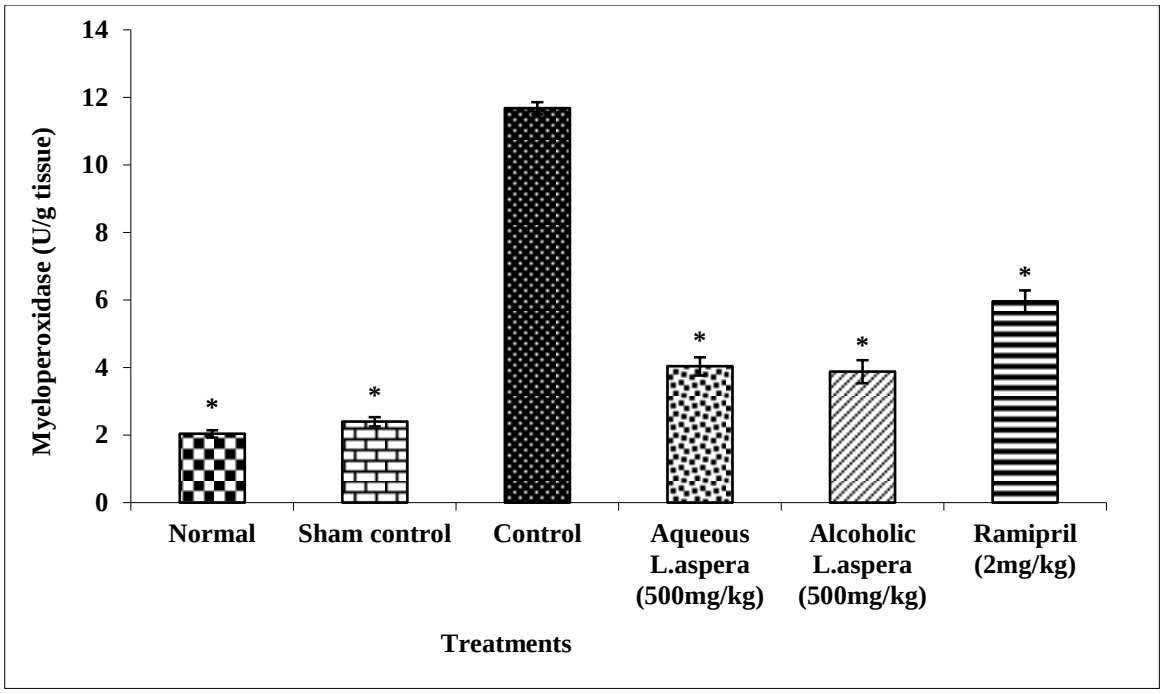

Figure 6: Effect of aqueous and alcoholic extracts of *Leucas aspera* on myeloperoxidase induction in experimental group animals

All values are expressed as Mean ± S.E.M. of five animals. *P<0.001, statistically significant compared to control group.

DISCUSSION

Reperfusion of ischemic myocardium is necessary to salvage tissue from eventual death. In order to restore coronary flow, percuataneous transluminal coronary angioplasty, percuataneous transluminal coronary recanalization or stenting has been applied to patients with acute myocardial infarction [40, 41]. However, the prognosis is not always excellent even if coronary reperfusion is achieved completely. Reperfusion itself causes myocardial dysfunction, which has been recognized as ischemia-reperfusion injury based on the findings of animal experiments [42, 43].

Reperfusion injury is the result of complex interactions between substances accumulating during ischemia and those delivered following reperfusion. In ischemia-reperfusion of the heart, oxygen free radicals are thought to play an important role in the genesis of tissue injury [44, 45]. These free radicals can initiate the formation of alkyl, alkoxy and hydroperoxy radicals plus hydroperoxides from polyunsaturated fatty acids in membrane makes the latter vulnerable to free radical induced lipid peroxidation. In the present investigation we observed a significant elevation in lipid peroxide levels in serum and heart tissue and significant increase in infarct size in the control group as compared to sham control group. In groups treated with aqueous and alcoholic extracts ameliorated the elevated lipid peroxide levels in serum and heart tissue and significant reduction in infarct size in a dose dependent manner. The reference drug ramipril produced decrease in lipid peroxide levels in serum and heart tissue and significantly reduced the infarct size compared to control group.

Myocardial ischemia-reperfusion resulted in an increase in the level of free radicals, which inturn, induce cellular damage and this observation could be sustained by the low levels of free radical scavenging enzymes such as SOD, CAT, GSH and GST, that formed the first line of cellular defense against the oxidation injury. SOD and CAT are important antioxidant enzymes in mitigating free radical induced cell injury. Glutathione implicated in removal of free oxygen species such as hydrogen peroxide, superoxide radicals alkoxy radicals and maintenance of membrane protein thiols and as substrate for glutathione peroxidase and glutathione-S-transferase. A decrease in the activity of superoxide dismutase and catalase could result in the decreased removal of superoxide ion and hydrogen peroxide radicals which brings about a number of reactions which are harmful to myocardium. In the present study, ischemia-reperfusion injury was associated with increased oxidative stress was evidenced by the decreased levels of endogenous antioxidant enzymes SOD, CAT, GSH and GST in heart tissue and the results were significantly comparable with sham control animals. Indeed, both alcoholic and aqueous extracts proved beneficial in restoring the levels of these endogenous antioxidant enzyme levels in a dose dependent manner. The rise in myocardial SOD, CAT, GSH and GST levels were observed with both aqueous and alcoholic extracts at doses 250, 500 mg/kg, but not with 100 mg/kg dose compared to control group. In ramipril treated group, myocardial SOD, GSH and GST were restored.

Besides antioxidant enzymes, alteration in cytosolic enzymes such as SGOT, SGPT LDH, CK-MB and CK, has been considered as an important diagnostic markers of myocardial infarction. These cytosolic enzymes leaked out from the tissue to plasma on development of degenerative changes in myocardial cell membrane. In the present study, marked elevation in the levels of these enzymes in the serum of control group suggests the occurrence of considerable membrane damage

when compared to sham control group. Treatment with aqueous and alcoholic extracts of *L. aspera* resulted in significant reduction in the levels of these enzymes in a dose dependent manner compared to control group.

Neutrophils represent a potentially important source of oxygen free radicals, myeloperoxidase activity as a marker for the infiltration of neutrophils into inflamed tissue. In the present study, the MPO levels are significantly elevated in control group when compared to sham control group. In groups treated with aqueous and alcoholic extracts, the reduced levels of myeloperoxidase indicated that pretreatment with aqueous and alcoholic extracts suppressed neutrophil infiltration into the injured myocardium.

CONCLUSION

The present finding suggests that both aqueous and alcoholic extract of *Leucas aspera* posses a dose dependent cardioprotection against ischemia-reperfusion induced myocardial injury. Reduction in infarct size and decreased levels of lipid peroxides in serum and heart tissue, and decreased levels of MPO clearly indicates that *L. aspera* protects the loss of membrane integrity and stabilizing the membrane by neutralizing the cytotoxic free radicals generated during I/R injury thereby protects the myocyte from oxidative stress. The cardioprotective activity of *L. aspera* is further confirmed by antagonizing the decrease in antioxidant enzymes and rise in serum marker enzymes during the ischemia-reperfusion injury.

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