Aqueous extract of *Saussurea lappa* root ameliorate oxidative myocardial injury induced by isoproterenol in rats

T. S. Mohamed Saleem,
N. Lokanath, A. Prasanthi,
M. Madhavi, G. Mallika,
M. N. Vishnu

Department of Pharmacology,
Annamacharya College of Pharmacy,
Rajampet, Andhra Pradesh, India

J. Adv. Pharm. Tech. Res.

ABSTRACT

Saussurea lappa Clarke (Compositae), is commonly known as Kushta. In Ayurvedha, it is mentioned that the aqueous extract of the root *S. lappa* was used for treatment of angina pectoris. The present study was designed to investigate the cardioprotective effect of aqueous extract of root of *S. lappa* against isoproterenol induced myocardial injury. Myocardial injury in rat was induced by the administration of isoproterenol at a dose of 85 mg/kg, i.p., The rats were pretreated with the aqueous extract of *S. lappa* (AESL) in three different doses (100, 200 and 300 mg/kg, p.o.) through the oral route. Isoproterenol alone-treated rats showed increased serum concentration of lactate dehydrogenase (LDH), creatinine kinase (CK), and aspartate transaminase (AST), increased myocardial thiobarbituric acid reactive substances (TBARS) level, and decreased myocardial glutathione (GSH) level due to myocardial damage produced by isoproterenol. This is further conformed by histopathological changes. Chronic oral administration of AESL in three different doses significantly restored the level of myocardial LDH, CK, AST, TBARS, and GSH. The extract effect was compared with the reference standard α-tocopherol which also offered similar protection in biochemical and histopathological changes. The overall beneficial effect which was observed with the dose of 200 mg/kg indicated that AESL produced significant dose-dependent activity against isoproterenol induced myocardial injury.

Key words: Antioxidants, lactate dehydrogenase, myocardial infarction, oxidative stress, *Saussurea lappa*

INTRODUCTION

Cardiovascular disease (CVD) is known as a life-threatening problem with high mortality and morbidity at the global level. According to the World Health Organization report, the maximum mortality occurs within the age limit of 40-50 years due to myocardial infarction (MI) in both western and developing countries.[1-3] Oxidative stress due to extensive generation of free radicals with concomitant depletion of endogenous antioxidants like superoxide dismutase, catalase and reduced glutathione (GSH) play an important role in MI.[3]

Natural antioxidants play a major role in reducing the oxidative stress by scavenging the excess free radicals.[4] Saussurea lappa is one of the antioxidant-rich medicinal plants. S. lappa Clarke (Compositae), commonly known as Kushta in Sanskrit, is a tall, robust, perennial herb distributed in Kashmir. The hot water extract of the roots has been traditionally used for the treatment of asthma,[5,6] inflammations, and rheumatism.[5,7] The roots are hot, bitter, sweetish, pungent, and flattering. It is used as an analgesic, digestive, aphrodisiac and diuretic. Many authors have reported that the roots of this plant possess cortisol-lowering effect, bronchodilator, antiulcer, anticancer, anti-inflammatory, antiviral, and hepatoprotective effects.[8,9] Traditionally, aqueous extract

Access this article online	
Quick Response Code:	**Website:** www.japtr.org
	DOI: 10.4103/2231-4040.111525

of the root of *S. Lappa* was used for its anti-anginal effect.[10] So, the present research has been designed to evaluate the cardioprotective property of the aqueous extract of *S. Lappa* (AESL) root to support the traditional claim.

MATERIALS AND METHODS

Chemicals
Isoproterenol hydrochloride, thiobarbituric acid, 2,4 dinitrophenyl hydrazine, and GSH were purchased from Sigma Chemical, Bangalore. Biochemical kits for lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate transaminase (AST) were purchased from Transasia Bio-Medicals Limited, Solan. All other reagents and chemicals used in this study were of analytical grade with high purity.

Animals
Male Wistar albino rats weighing 200-300 g were selected for the study. The animals were housed in their respective cages under hygienic and standard environmental conditions (28 ± 2°C, humidity 60-70%, 12-h light and dark cycle). The animals were allowed a standard feed (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum.* They were acclimatized to the environment for 1 week prior to experimental use. The study protocol was carried out after obtaining the permission from the Institutional Animal Ethical Committee (1220/a/08/CPCSEA).

Plant Material and Preparation of Extract
The root powder of *S. lappa* was purchased from an Indian druggist, Tirupati and authenticated by Dr. Madhava Chetty, Professor and Head, Department of Botany, S.V. University, Tirupathi. About 1 kg of the powdered material was boiled with 5 l of distilled water for 30 min and filtered to obtain the aqueous extract. The extract was concentrated under reduced pressure and lyophilized. The freeze-dried material was weighed (about 35 g), dissolved in water (at a final concentration of 50 mg/ml) and used for this study.

Preliminary Phytochemical Analysis
AESL was analyzed for the various classes of phytoconstituents such as flavonoids, phenolic acids, anthocyanins, quinones, alkaloids, tannins, and saponins using standard phytochemical methods.[11]

Estimation of Phenolics
Phenolic content of AESL was determined by the method of Malick and Singh, 1980.[12] Briefly, an aliquot of the sample was pipetted out in a test tube and the volume was made up to 3 ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) was added to the tube and incubated for 3 min. at room temperature. Sodium carbonate (20%; 2 ml) solution was added, mixed thoroughly, and the tube was incubated for 1 min in boilng water bath. The absorbance was measured at 650 nm against a reagent blank. Standard curve using different concentrations of standard phenolic-catechol was prepared. From the standard curve, concentration of phenol in the test sample was determined and expressed as mg of catechol equivalent.

Estimation of Flavonoids
The flavonoid content of AESL was determined by the method of Helmja *et al.*[13] Briefly, an aliquot of the sample was pipetted out in a test tube and the volume was made up to 0.5 ml with distilled water. Sodium nitrite (5%; 0.03 ml) was added to the tube and incubated for 5 min. at room temperature. Aluminum chloride solution (10%; 0.06 ml) was added and incubated for 5 min. at room temperature. Sodium Hydroxide solution (1 M; 0.2 ml) was added and the total volume was made up to 1 ml with distilled water. Absorbance was measured at 510 nm against a reagent blank. Standard curve using different concentrations of rutin was prepared. From the standard curve, the concentration of flavonoids in the test sample was determined and expressed as mg of rutin equivalent.

In-vitro Antioxidant Activity of Aqueous Extract of *Saussurea Lappa*
Determination of reducing power
The reducing power of the AESL was determined by the method described by Oyaizu (1986).[14] Different concentrations of the AESL and ascorbic acid were prepared and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50°C for 20 min, 2.5 ml of 10% TCA was added to the blend and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was assorted with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the reducing power.

2,2-diphenyl-1-picrylhydrazyl assay
The hydrogen atom or electron-donating group of the resultant compounds and some untainted compounds was measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical, DPPH as a reagent.[15] One ml of different concentrations of the AESL and ascorbic acid in ethanol were added to 4 ml of 0.004% methanol solution of DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in the following way: $I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$ where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of extract.

Determination of peroxide (H_2O_2) radical-scavenging activity
The peroxide (H_2O_2) radical-scavenging activity of AESL

was determined by the method described by Ruch *et al.*,[16] 1 ml of sample solution (AESL and ascorbic acid in various concentrations prepared in phosphate-buffered saline [PBS]) was incubated with 0.6 ml of 4 mM H_2O_2 solution (prepared in PBS) for 10 min. The absorbance of the solution was read at 230 nm against a blank solution containing the extract without H_2O_2. The concentration of H_2O_2 was spectrophotometrically stubborn from absorption at 230 nm using the molar absorptivity of 81/M/cm. The H_2O_2 radical-scavenging activity was calculated as $1\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of control and A_{sample} is the absorbance of test.

Acute Oral Toxicity Study

The acute oral toxicity study was performed according to up and down procedure. AESL up to a dose of 2000 mg/kg did not produce any signs of toxicity and mortality.

Induction of Myocardial Infarction

The MI was induced in experimental rats by intraperitoneal injection of isoproterenol hydrochloride 85 mg/kg body weight, dissolved in physiological saline, for 2 consecutive days.[17]

Experimental Protocol

The rats were randomly divided into six groups with six rats in each group. Group I, normal animals received saline 10 ml/kg bw with standard feed and water to allowed *ad libitum* throughout the experimental period. Group II, the rats were orally fed normal saline once daily for 28 days and in addition, received isoproterenol (85 mg/kg body weight) on the 29 and 30 day at an interval of 24 h. Group III-V, rats were pretreated with AESL (100, 200 and 300 mg/kg body weight respectively) for a period of 28 days and in addition, received isoproterenol 85 mg/kg body weight) on the 29 and 30 day at an interval of 24 h. Group VI, rats were pretreated with α-tocopherol (60 mg/kg body weight, orally) for a period of 28 days and in addition, received isoproterenol 85 mg/kg body weight) on the 29 and 30 day at an interval of 24 h.[17]

At the end of the treatment period, blood samples were collected by the retro-orbital plexus puncture method under light ether anesthesia and serum was separated by centrifugation and used for the biochemical estimations (LDH, CK and AST) using the respective kits. The heart was excised immediately and immersed in physiological saline. It was suspended in 10% (w/v) ice-cold 0.1M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed and homogenized using a Teflon homogenizer (Inco, India). The clear supernatant was used for estimation of endogenous antioxidant enzymes such as reduced GSH[18] and thiobarbituric acid reactive substances (TBARS)[19] by a spectrophotometer.

Histological Examinations

The hearts were removed, washed immediately with ice-cold saline; then fixed in 10% buffered formalin; 10% stored buffered formalin were embedded in paraffin; 5-μm thick sections were cut and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

Statistical Analysis

Values are expressed as mean ± SD and analyzed using Graph Pad Prism Version 5.1 using ANOVA followed by Tukey's multiple comparison Test. $P < 0.05$ was considered significant.

RESULTS

Phytochemical Investigation

Preliminary phytochemical investigation of AESL revealed the presence of flavonoids, terpenoids, alkaloids, and phytosterols as important active constituents. Phenolic and flavonoid content of AESL were determined and the results indicated that AESL contains 3.5 mg/g of catechol-equivalent phenolics and 16.0 mg/g of rutin-equivalent flavonoid.

In-vitro Antioxidant Activity

In the present study, increasing the concentration of AESL and ascorbic acid shows increased absorbance, indicating the reducing power. The reducing power of AESL was very potent but lower than that of ascorbic acid and the reducing power of the AESL was increased with the quantity of sample [Figure 1]. AESL and ascorbic acid show DPPH-inhibition activity in different concentrations. And in higher concentration (500 μg), AESL shows higher % inhibition activity (100%) and when compared with ascorbic acid (100%), it was equal. The IC_{50} value of AESL and ascorbic acid is found to be 70, 120 μg ml respectively [Figure 2]. AESL and ascorbic acid show H_2O_2-inhibition activity in different concentration. While increasing concentration, the % inhibition activity also increased. When compared with ascorbic acid, the % inhibition activity is low in AESL. The IC_{50} value of AESL and ascorbic acid is found to be 150, 120 μg ml respectively [Figure 3].
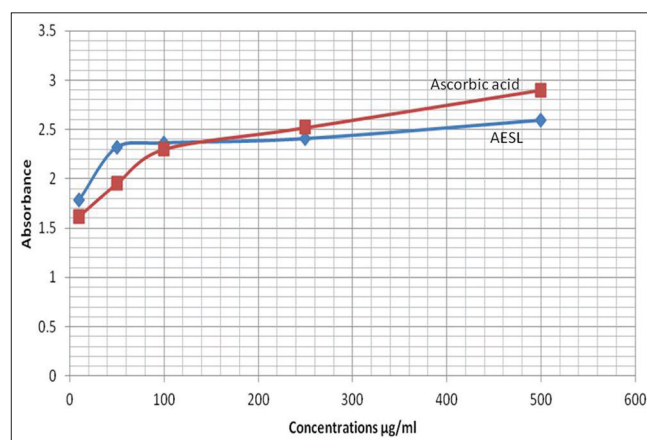

Figure 1: Reducing power of aqueous extract of *saussurea lappa*

In acute toxicity study, it was found that the animals were safe up to a maximum dose of 2,000 mg/kg b.w. There were no changes in the normal behavioral pattern and no signs and symptoms of toxicity and mortality were observed. The results obtained in the different groups subjected to ISO-induced ischemic injury are presented in Tables 1-3.

Myocardial and Serum Lactate Dehydrogenase

Myocardial LDH in Group II was significantly ($P < 0.001$) lower than that in Group I. In Group III-VI the level of LDH was restored to normal when compared with Group II. There was a significant ($P < 0.001$) increase in the level of serum LDH in Group II when compared with Group I and the level significantly decreased in the Groups III ($P < 0.001$), IV ($P < 0.001$), V ($P < 0.001$), and VI ($P < 0.001$).

Myocardial and Serum Creatinine Kinase

Myocardial CK in Group II was significantly ($P < 0.001$) lower than that in the control group, i.e., Group I. In Groups III-VI, the level of CK was restored to normal when compared with Group I. There was significant ($P < 0.001$) increase in the level of serum CK in Group II when

compared with control Group I and the level significantly decreased in the Groups III ($P < 0.001$), IV ($P < 0.001$), V ($P < 0.001$), and VI ($P < 0.001$).

Myocardial and Serum Aspartate Transaminase

There were no significant changes in the level of myocardial

Table 1: Serum level of lactate dehydrogenase, creatinine kinase and aspartate transaminase

Treatment group	LDH U/l	Creatinine kinase U/l	AST U/l
Control (Saline 10 ml/kg)	29.8±5.8	58.4±20.2	27.7±1.3
Isoproterenol (85 mg/kg)	139.7±10.1***	264.7±23.7***	95.4±23.2***
AESL (100 mg/kg)	46.6±35.3***	86.3±73.6***	50.8±13.4***
AESL (200 mg/kg)	34.7±5.3***	64.1±20.6***	65.4±7.7**
AESL (300 mg/kg)	37.7±7.7***	142.3±23.5***	51.8±5.9***
α-tocopherol (60 mg/kg)	55.8±33.4***	72.3±18.5***	40.6±5.8***

All value expressed as mean±SD; One-way analysis of variance followed by Tukey's multiple comparison test, ***$P<0.001$,**$P<0.01$, LDH: Lactate dehydrogenase, AST: Aspartate transaminase, AESL: Aqueous extract of *Saussurea lappa*

Table 2: Myocardial tissue level of lactate dehydrogenase, creatinine kinase and aspartate transaminase

Treatment group	LDH U/l	Creatinine kinase U/l	AST U/l
Control (Saline 10 ml/kg)	37.6±10.3	130.8±6.8	69.6±4.1
Isoproterenol (85 mg/kg)	5.9±1.4***	43.4±25.6***	62.5±2.3[ns]
AESL (100 mg/kg)	38.7±7.7***	152.3±31.4***	68.1±16.5[ns]
AESL (200 mg/kg)	35.9±6.9**	192.9±5.9***	77.2±0.77[ns]
AESL (300 mg/kg)	37.3±19.2***	196.2±43.8***	87.6±17.6**
α-tocopherol (60 mg/kg)	29.9±14.9*	110.9±9.3***	83.6±9.1*

All value expressed as mean±SD; One-way analysis of variance followed by Tukey's multiple comparison test, ***$P<0.001$, **$P<0.01$, *$P<0.05$, Ns: Non-significant, LDH: Lactate dehydrogenase, AST: Aspartate transaminase, AESL: Aqueous extract of *Saussurea lappa*

Figure 2: 2,2-diphenyl-1-picrylhydrazyl inhibition activity of aqueous extract of *saussurea lappa*

Figure 3: Hydrogen peroxide radical scavenging activity of aqueous extract of *saussurea lappa*

Table 3: Myocardial tissue level of thiobarbituric acid reactive substances and glutathione

Treatment group	TBARS nmol/g wet wt	GSH µg/g wet wt
Control (Saline 10 ml/kg)	31.8±1.2	138.4±0.71
Isoproterenol (85 mg/kg)	103.2±29.5**	85.2±14.3*
AESL (100 mg/kg)	48.6±31.6[ns]	118.5±12.2[ns]
AESL (200 mg/kg)	23.5±4.6**	198.1±4.4***
AESL (300 mg/kg)	66.6±4.5[ns]	244.8±5.1**
α-tocopherol (60 mg/kg)	20.1±4.9**	188.2±9.6***

All value expressed as Mean±SD; One-way analysis of variance followed by Tukey's multiple comparison test, ***$P<0.001$, **$P<0.01$, *$P<0.05$, Ns: Non-significant, TBARS: Thiobarbituric acid reactive substances, AESL: Aqueous extract of *Saussurea lappa*, GSH: Glutathione
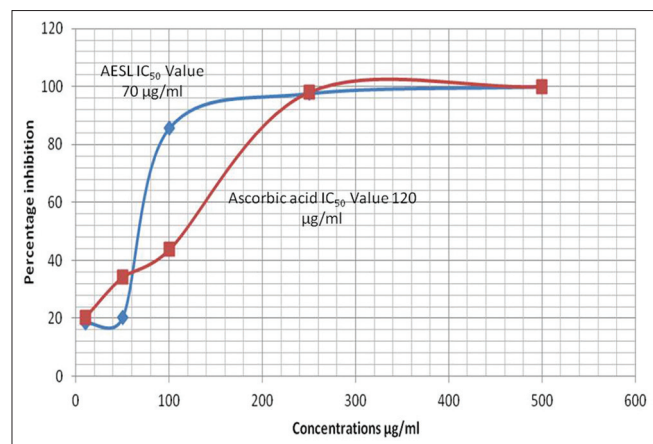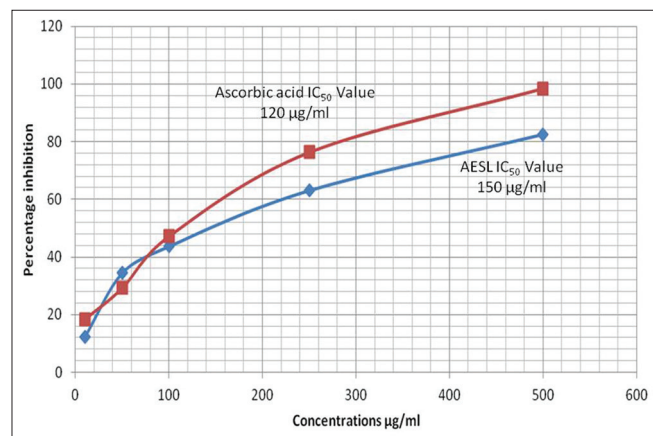
AST in Group II and Group III-IV when compared with Group I and II respectively. In Group V and VI there was a significant ($P < 0.01$ and $P < 0.05$ respectively) increase in the level of AST than that in Group II. There was a significant ($P < 0.001$) increase in the level of serum AST in Group II when compared with control Group I and the level significantly decreased in the Groups III ($P < 0.001$), IV ($P < 0.01$), V ($P < 0.001$), and VI ($P < 0.001$).

Myocardial Thiobarbituric Acid Reactive Substances

Myocardial TBARS in Group II was significantly ($P < 0.01$) higher than that in control Group I. In Groups IV and VI, there was a significantly ($P < 0.01$) lower TBARS level in comparison to Group II. There were no significant changes in the level of TBARS in Group III and V when compared with Group II.

Myocardial Glutathione

There was significant ($P < 0.05$) decrease in the level of GSH in Group II when compared with Group I. The level of GSH was significantly increased in the Groups III and VI ($P < 0.001$) and Group V ($P < 0.01$) when compared to Group II. There were no significant changes in the level of GSH in Group III when compared with Group II.

Histological Changes

Light microscopy of the tissue sections of Group I showed the normal cyto architecture of the heart tissue. Group II showed the necrotic changes in myocardial tissue. The tissue sections of both the Groups III-IV showed regenerative changes in

heart tissue and Group V-VI showed normal architecture of the heart tissue. The results are presented in Figure 4.

DISCUSSION

Isoproterenol is a well-known cardiotoxic agent due to its ability to destroy myocardial cells. As a result of this, cytosolic enzymes such as LDH, AST, and CK were released into the blood stream and serve as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in heart reflects the alterations in plasma membrane integrity and/or permeability.[20] Changes in the level of myocardial markers LDH and CK in both serum and heart homogenate in ISO-treated rats [Tables 1 and 2] confirms the onset of myocardial necrosis.[21] Chronic oral administration of AESL in three different doses (100, 200, and 300 mg/kg p.o) caused significant change in the level of cardiac markers (LDH, CK, and AST) in both serum and myocardium. But, there was no significant alteration in the level of myocardial AST in ISO-treated and AESL (100 and 200 mg/kg) groups and AESL in higher dose (300 mg/kg) significantly increased the level of myocardial AST when compared with baseline level (Group I).

Administration of ISO generates the free radicals in the myocardium through oxidative stress and produces myocardial necrosis.[22] The principal finding of the present study is that ISO-induced myocardial necrosis was associated with oxidative stress, as evidenced by increase in myocardial
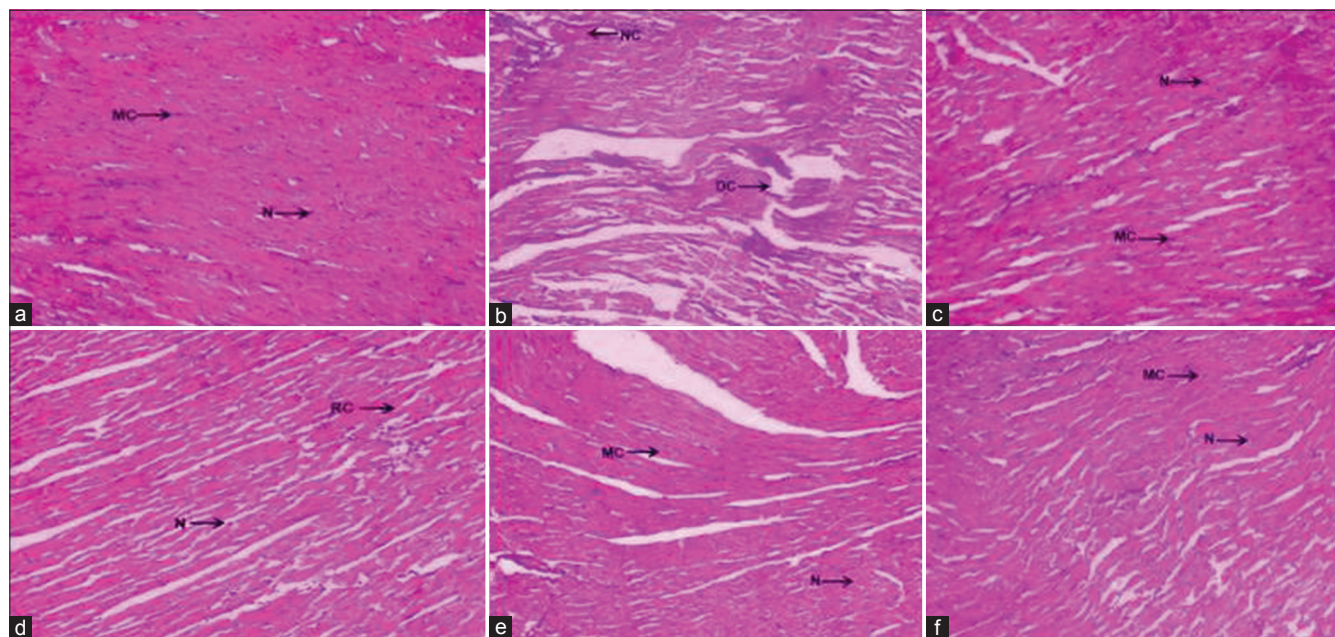

Figure 4: Histopathological report. (a) Group I: Vehicle-received rat heart shows the normal cyto-architecture of the myocardium. (b) Group II: ISO-treated rat heart shows the necrotic changes in myocardial tissue. (c) Group III: Aqueous extract of *saussurea lappa* 1 (100 mg/kg)-treated rat heart shows regenerative changes in myocardial tissue. (d) Group IV: Aqueous extract of *saussurea lappa* 2 (200 mg/kg)-treated rat heart shows regenerative changes in myocardial tissue. (e) Group V: SL (300 mg/kg)-treated rat heart shows normal cyto-architecture of myocardium. (f) Group VI: α-tocopherol (60 mg/kg)-treated rat heart shows normal cyto-architecture of myocardium. N=Nucleus; ID=Intercalated disks; NC= Necrotic changes; MC= Myocardial cells; DC= Degenerative changes; RC= Regenerative changes

TBARS in Group II [Table 3]. Similar observations were made earlier by other studies.[23-27] Chronic oral administration of AESL prevents the oxidative stress and the structural changes associated with ISO-induced myocardial necrosis. The mechanism of such protection by the chronic oral administration of AESL may be due to myocardial adaptation, oxidative stress is mediated through reduction in the TBARS level.[28] In the present study, we observed that AESL at 200 mg/kg only significantly reduced the oxidative stress and lower (100 mg) or higher (300 mg) doses do not offer significant protection against oxidative stress [Table 3].

Antioxidants play a vital role in eliminating the reactive oxygen species. GSH is one of the major antioxidant enzymes to scavenge the free radicals during tissue damage.[24] GSH scavenges singlet oxygen, superoxide, and peroxy radicals to form oxidized GSH and other disulfides. Also, antioxidant compounds have been shown to increase GSH reductase activity, that maintains GSH in a reduced state.[23,25] Decrease in the level of GSH in ISO-treated animals [Table 3] indicated that the depletion of GSH resulted in enhanced lipid peroxidation, and excessive lipid peroxidation caused increased GSH consumption.[23] AESL-treated groups showed that the significant increase in the level of GSH may be due to its enhanced synthesis. But, this effect is not observed with the lower dose (100 mg) of AESL [Table 3]. Cardio protective effects of AESL was compared with α-tocopherol as the standard natural antioxidant also offered significant protection against ISO-induced depletion of marker enzymes and oxidative stress. This action may be probably due to suppression of membrane damage and reduction in membrane fluidity. Light microscopy examination of rat heart-section treated with AESL and α-tocopherol restored the myocardial damage with no evidence of focal damage produced by isoproterenol, which showed the cytoprotective action of AESL [Figure 4].

Administration of antioxidant-rich natural drugs decreases the mortality from CVD and also promises a therapeutic approach to combat oxidative stress associated with cardiac diseases.[3] AESL also offered significant inhibition activity in *in-vitro* antioxidant model which confirmed the protective role via the antioxidant mechanism. As per phytochemical investigation, the AESL contain flavonoids and phenolic compounds in high concentrations, which might be a responsible active principle for the cardio protective action.

CONCLUSION

In summary, it has been concluded from the biochemical and histopathological evidence that the AESL produced significant dose-dependent cardioprotection in isoproterenol-induced MI. This is the first report to support the ayurvedic recommendation and further study is ongoing to isolate and elucidate the mechanism of action of the active principle.

REFERENCES

1. Panwar RB, Gupta R, Gupta BK, Raja S, Vaishnav J, Khatri M, *et al*. Atherothrombotic risk factors and premature coronary heart disease in India: A case-control study. Indian J Med Res 2011;134:26-32.
2. Kumar A, Khan SA, Parvez A, Zaheer MS, Rabbani MU, Zafar L. The prevalence of hyperhomocysteinemia and its correlation with conventional risk factors in young patients with myocardial infarction in a tertiary care centre of India. Biomed Res 2011;22:225-9.
3. Saleem TS, Chetty M, Kavimani S. Sesame oil enhances endogenous antioxidants in ischemic myocardium of rat. Rev Bras Farmacogn 2012;22:669-75.
4. Ahmad S, Khan MB, Hoda MN, Bhatia K, Haque R, Fazili IS, *et al*. Neuroprotective effect of sesame seed oil in 6-hydroxydopamine induced neurotoxicity in mice model: Cellular, biochemical and neurochemical evidence. Neurochem Res 2012;37:516-26.
5. Shah NC. Herbal folk medicines in Northern India. J Ethnopharmacol 1982;6:293-301.
6. Sircar NN. Pharmaco-therapeutics of dasemani drugs. Anc Sci Life 1984;3:132-5.
7. Lechner-Knecht S. Sacred healing plants in Nepal. Dtsch Apoth Ztg 1982;122:2122-9.
8. Chen HC, Chou CK, Lee SD, Wang JC, Yeh SF. Active compounds from *Saussurea lappa* Clarks that suppress hepatitis B virus surface antigen gene expression in human hepatoma cells. Antiviral Res 1995;27:99-109.
9. Ambavade SD, Mhetre NA, Tate VD, Bodhankar SL. Pharmacological evaluation of anxiolytic effect of aqueous extracts of *Saussurea lappa* roots in mice. Eur J Integr Med 2009;1:131-7.
10. Khare CP. *Saussurea lappa*. Indian Medicinal Plants: An Illustrated Dictionary. Berlin: Sringer-verlag; 2007. p. 586-7.
11. Harbone JB, Baxter HH. Phytochemical Dictionary: A hand Book of Bioactive Compound from plants. Washington: Taylor and Francis; 1993.
12. Malick CP, Singh MB. In: Plant Enzymology and Histoenzymology. New Delhi: Kalyani Publishers; 1980. p. 286.
13. Helmja K, Vaher M, Gorbatšova J, Kaljurand M. Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. Proc Estonian Acad Sci Chem 2007;56:172-86.
14. Oyaizu M. Studies on products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44:307-15.
15. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res 2000;14:323-8.
16. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10:1003-8.
17. Gauthaman KK, Saleem MT, Thanislas PT, Prabhu VV, Krishnamoorthy KK, Devaraj NS, *et al*. Cardioprotective effect of the Hibiscus rosa sinensis flowers in an oxidative stress model of myocardial ischemic reperfusion injury in rat. BMC Complement Altern Med 2006;6:32.

18. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.

19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.

20. Thippeswamy BS, Patil U, Thakker SP, Desai S, Tubachi S, Kalyani GA, *et al*. Cardioprotective effect of cucumis trigonus roxb on isoproterenol-induced myocardial infarction in rat. Am J Pharmacol Toxicol 2009;4:29-37.

21. Ithayarasi PA, Devi CS. Effect of α-tocopherol on isoproterenol induced changes in lipid and lipoprotein profile in rats. Ind J Pharmacol 1997;29:399-404.

22. Trivedi CJ, Balaraman R, Majithiya JB, Bothara SB. Effect of atorvastatin treatment on isoproterenol-induced myocardial infarction in rats. Pharmacology 2006;77:25-32.

23. Karthikeyan K, Sarala Bai BR, Niranjali Devaraj S. Grape seed proanthocyanidins ameliorates isoproterenol-induced myocardial injury in rats by stabilizing mitochondrial and lysosomal enzymes: An *in vivo* study. Life Sci 2007;81:1615-21.

24. Kuppusamy A, Subhadradevi V, Christy J, Honey J, Daphne S, Divia C, *et al*. Cardioprotective effect of Erythrina stricta leaves on isoproterenol-induced myocardial infarction in rat. Bangladesh J Pharmacol 2010;5:1-4.

25. Thounaojam MC, Jadeja RN, Ansarullah, Karn SS, Shah JD, Patel DK, *et al*. Cardioprotective effect of Sida rhomboidea. Roxb extract against isoproterenol induced myocardial necrosis in rats. Exp Toxicol Pathol 2011;63:351-6.

26. Murugesan M, Revathi R, Manju V. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Indian J Pharmacol 2011;43:516-9.

27. Ojha S, Bharti S, Golechha M, Sharma AK, Rani N, Kumari S, *et al*. Andrographis paniculata extract protect against isoproterenol-induced myocardial injury by mitigating cardiac dysfunction and oxidative injury in rats. Acta Pol Pharm 2012;69:269-78.

28. Das DK, Maulik N, Moraru II. Gene expression in acute myocardial stress. Induction by hypoxia, ischemia, reperfusion, hyperthermia and oxidative stress. J Mol Cell Cardiol 1995;27:181-93.

Author Help: Reference checking facility

The manuscript system (www.journalonweb.com) allows the authors to check and verify the accuracy and style of references. The tool checks the references with PubMed as per a predefined style. Authors are encouraged to use this facility, before submitting articles to the journal.

- The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a single spelling error or addition of issue number/month of publication will lead to an error when verifying the reference.
- Example of a correct style
  Sheahan P, O'leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. Otolaryngol Head Neck Surg 2002;127:294-8.
- Only the references from journals indexed in PubMed will be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum of 15 references at a time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.