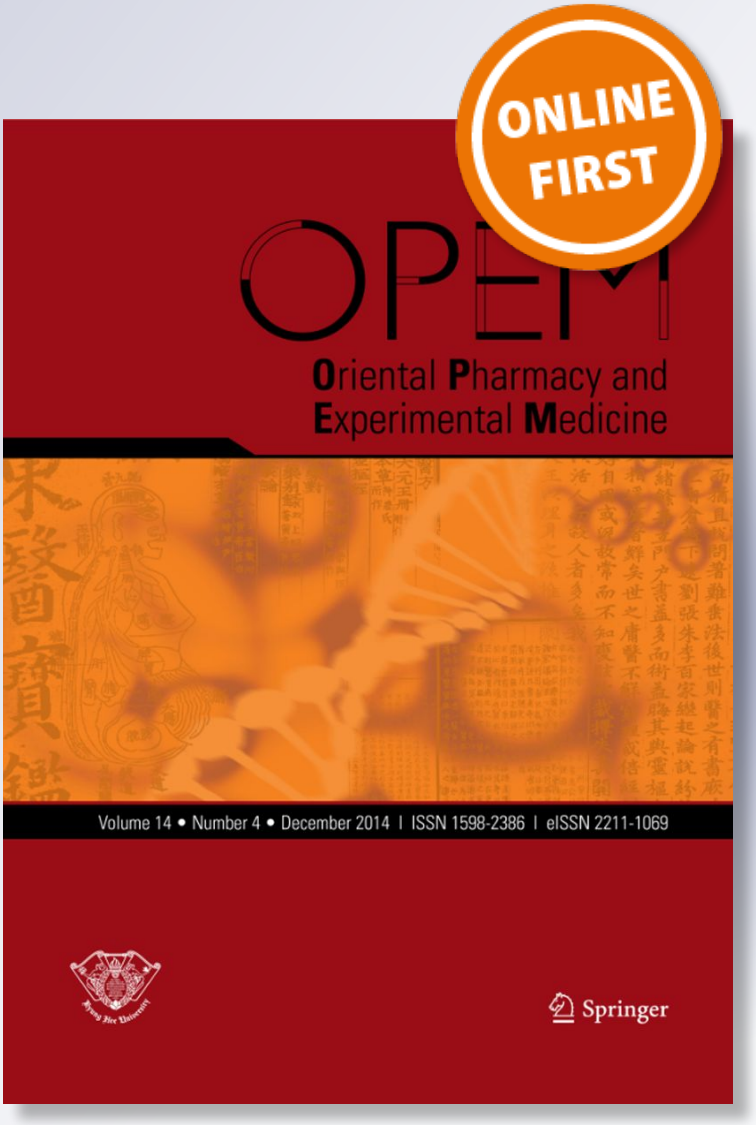
Rosa damascena *Mill. L. attenuates myocardial lysosomal membrane destabilization in isoproterenol induced oxidative stress*

Chitikela P. Pullaiah, G. V. Narasimha Kumar, K. Jyothsna, K. Thyagaraju, Vinod K. Nelson & G. Dayanand Reddy

Oriental Pharmacy and Experimental Medicine

ISSN 1598-2386

Orient Pharm Exp Med
DOI 10.1007/s13596-017-0290-x



Springer

Orient Pharm Exp Med
https://doi.org/10.1007/s13596-017-0290-x

Online ISSN 221
Print ISSN 1598-2386

RESEARCH ARTICLE

Rosa damascena Mill. L. attenuates myocardial lysosomal membrane destabilization in isoproterenol induced oxidative stress

Chitikela P. Pullaiah[1,2] · **G. V. Narasimha Kumar**[1] · **K. Jyothsna**[3] · **K. Thyagaraju**[2] · **Vinod K. Nelson**[4] · **G. Dayanand Reddy**[1]

Received: 30 May 2017 / Accepted: 11 October 2017

Abstract Lysosomal membrane damage suggests that it would be the major contributing factor of myocardial infarction followed by necrosis. The present investigation was carried out on the defensive effect of ethanolic extract of *Rosa damascena* Mill. L. extract on alterations in myocardial lysosomal enzyme activity and membrane bound Na^+/K^+ ATPases against isoproterenol induced myocardial infarction in rats. On treatment with isoproterenol (100 mg/kg body weight) for two consecutive days, animals have shown a significant increase in serum creatinine kinase-MB, lactate dehydrogenase and lysosomal enzyme activity; whereas membrane bound Na^+/K^+ ATPases and tissue antioxidants were decreased. Pre-treatment with Rosa *damascena* Mill. L. extracts 200 and 400 mg/kg body weight respectively, for 28 days, significantly prevented the alterations and restored the CK-MB, LDH, tissue antioxidants and lysosomal enzyme activity to near-normal status in rats. These findings demonstrate that *Rosa damascena* could preserve lysosomal integrity through increasing the antioxidant enzyme levels and membrane bound Na^+/K^+ ATPases integrity. The cardio protective activity of *Rosa damascena* was further supported by histopathological examination of heart sections. Hence all these findings confirm cardio protective potential of ethanolic extract of *Rosa damascena*.

Keywords Antioxidant · Cardioprotection · Isoproterenol · Oxidative stress · Lysosomal enzyme

Introduction

According to the World Health Organization (WHO), cardiovascular diseases are the world's largest killer, claiming the lives of at least 17.1 million persons each year (Gadham et al. 2012). Myocardial infarction (MI) results from any interruption in the blood supply to heart, and leads to the death of the cardiac tissue, myocardial necrosis. The consequences of MI include hyperlipidemia, peroxidation of membrane lipids, and loss of plasma membrane integrity (Ahmed et al. 2014). Isoproterenol-induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function (Daniela et al. 1998). Catecholamine administration lead to complex biochemical and structural changes that cause irreversible cellular damage, which is a prelude to necrosis (Rona et al. 1959). The highly reactive cytotoxic free radicals through auto-oxidation of catecholamines have been implicated as one of the important causative factors for isoproterenol (ISO) induced cardiac damage.

Lysosomal enzymes are important mediators of acute myocardial infarction and their release into the cytoplasm stimulate the formation of inflammatory mediators such as oxygen radicals and prostaglandins (Karthikeyan et al. 2007).

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13596-017-0290-x) contains supplementary material, which is available to authorized users.

✉ Chitikela P. Pullaiah
samuelpharma@gmail.com

[1] Department of Pharmacology, Siddha Central Research Institute, Central Council for Research in Siddha, Chennai 600106, India

[2] Department of Biochemistry, S.V. University, Tirupati 517502, India

[3] Department of Pharmacology, Sri Padmavathi School of Pharmacy, Tirupati 517503, India

[4] Department of Pharmaceutical Technology, Pharmacognosy and Phytotherapy Laboratory, Jadavpur University, Kolkata, West Bengal, India

Published online: 31 October 2017

Administration of ISO leads to a significant elevation of lysosomal enzyme activities in the serum and myocardium. The study proposed by Kalra and Prasad., states that the oxygen free radicals generated during ischemia, in addition to the direct myocardial damaging effect, may also be responsible for the cardiac damage through the release of lysosomal enzymes (Kalra and Prasad 1994). It is possible that stabilization of myocardial cell membranes, particularly the lysosomal membranes, may prolong the viability of ischemic cardiac muscle and prevent MI (Ponnian et al. 2009).

Rosa damascena Mill. *L.* is common ornamental plant which is used in food industry, belonging to the family Rosaceae a well-known shrub cultivated in rose gardens in several places of central Asia and India. The therapeutic effects of *Rosa damascena* Mill. L. are due to its anti-inflammatory, analgesic, hypnotic, and antispasmodic properties. Antioxidant and antidiabetic, ionotropic agent, for the treatment of menstrual bleeding, antitussive, tracheal relaxant, and relaxing activity are the other effects that attributed to *Rosa damascena* Mill. *L.* (Mohammad et al. 2001).

The major background of the present study is, administration of ISO to liberate oxygen free radicals to lead to damage or destabilization, of lysosomal sacs in the cardiac myocytes and myocardium. The damaged lysosomes can release their hydrolyzing enzymes, which further augment the myocardial cell necrosis and infarction. The present study was designed to verify the hypothesis, that oxygen deprivation followed by free radical generation by ISO, associated with alterations in membrane bound Na^+/K^+ ATPase and myocardial lysosomal activity, we investigated the defensive effect of *Rosa damascena* Mill. L. extract on the above activity against ISO induced myocardial infarction in rats.

Materials and methods

Collection of plant parts

The flowers of *Rosa damascena* Mill. L. for the proposed study was collected in the month of January from fields of Tirupati, India and authentication was confirmed by Dr. N. Savithramma, Professor of Botany. S.V.University, Tirupati, India, and the specimen voucher in the form of herbarium (SVUH 1367) was deposited in the department.

Preparation of ethanolic extract of *Rosa damascena* (ERD)

The fresh petals of flower *Rosa damascena* Mill. L. was shade dried and ground to coarse powder. 250 g of coarse powder was subjected to cold maceration process using 70% ethanol as solvent. The extraction was continued for 3 days at room temperature with occasional shaking. Then the extract was filtered, and concentrated at 70 °C by using rotary flash evaporator and ethanolic extract of *Rosa damascena* Mill. L. (ERD) was obtained. The extract was lyophilized by freeze-dryer and stored at 4 °C for further use. The extract was diluted and used as and when required.

The preliminary phytochemical screening of ethanolic extract of *Rosa damascena* Mill. L. was carried out according to the methods described by Khandelwal et al. (2002).

Isoproterenol (CAS Number 5984–95-2), was purchased from Sigma Aldrich. Co, St. Louis, USA. All the chemicals used in the present study were of analytical grade and indigenous.

Animals

Male wistar rats weighed between 200 and 220 g were used in this study. Rats were housed under standard conditions and fed with standard pellet with drinking water ad libitum. The animals were kept in polypropylene cages and maintained at a room temperature of 25 ± 2^0c with 55 ± 5% relative humidity and 12 h light/dark cycle. All the experimental protocols were approved by the Institutional Animal Ethical Committee of Sri Padmavathi School of Pharmacy, Tirupati (SPSP/CPCSEA/IAEC-1016/a /2014/008).

Acute toxicity studies

An acute toxicity study was performed as per Organization for Economic Co-Operation and Development (OECD) 423 guidelines. Single doses of ethanolic extract of *Rosa damascena* Mill. L. ranging from 5, 50, 300 and 2000 mg/kg body weight were administered, separately. All the behavioral, motor and autonomic results were obtained as per Irvin scale and found no toxicity.

Induction of myocardial infarction

Myocardial infarction was induced, by dissolving isoproterenol (100 mg/kg) in normal saline and injected subcutaneously to rats for last two consecutive days of the experimental schedule (Periyathambi and Ponnian 2007).

Experimental schedule

The treatment schedule was fixed for 28 days, and the rats were divided into five groups of six each. Rats of group I received the normal saline and served as normal control, group II was received ISO (100 mg/kg body weight) for last two consecutive days of the study and served as disease control. Group III received metoprolol 10 mg/kg and serve as standard treatment, groups IV and V received 200 and 400 mg/kg body weight of ERD, respectively, once a day orally for 28 days of the study along with ISO for last two consecutive days of the study and serve as test groups.

Blood sample collection and analysis

At the end of treatment blood was collected from retro orbital plexus, by anesthetizing the rats with thiopental sodium (35 mg/kg body wt., intraperitoneally) (Prabhu et al. 2009) and serum was separated by centrifugation at 2000 rpm. Serum was used to analyze various biochemical parameters such as determinations of cardiac biomarkers lactate dehydrogenize (LDH), and creatinin kinase MB (CK-MB) by using commercial diagnostic kits (Agappe Pvt. Ltd., Kerala, India).

Na^+ /K^+ ATPase activity of myocardial membrane

The myocardial membrane Na^+ /K^+ ATPases activity was determined according to the procedure Periyathambi and Ponnian 2007. The incubation mixture contained 10 mM of Tris buffer, 20 mM of potassium chloride, 125 mM of sodium chloride, 1 mM of EDTA and 3 mM of ATP. To the incubation mixture, the reaction was initiated by the addition of 0.2 ml of tissue homogenate and the contents were incubated at 37 °C for 15 min. To stop the reaction of 10% TCA was added. The tubes were centrifuged and supernatant was used for the estimation of liberated P_i. 1.0 ml of supernatant was made up to 4.3 ml with distilled water and added 1.0 ml 3 mM of ammonium molybdate reagent. The tubes were incubated at room temperature for 10 min, and later 0.4 ml of amino napthol sulphonic acid reagent was added to develop the color and the P_i released recorded using a standard P_i graph.

Membrane stabilizing activity

Membrane stabilizing activity of ERD was performed using two separate methods, i.e., heat induced hemolysis and hypotonic solution induced hemolysis. Blood was collected from rats in a heparinized tube and washed with 0.9% saline for three times. Isotonic buffer solution (pH 7.4) was used to prepare a 40% (*v*/v) erythrocyte suspension, which was used to determine the membrane stabilization activities (Shinde et al. 1999).

Preparation of lysosomal sub cellular fractions

Lysosomal sub cellular fractions were isolated according to the method of Venkatachalem et al. The heart tissue sample was cut open and placed in isotonic saline to remove the blood. Then the heart tissue was rinsed in ice cold 0.25 M sucrose, blotted, weighed and minced. The enzyme extracts were prepared by homogenizing the tissue samples in 0.25 M sucrose at 4 °C. The portion of the homogenate was subjected to differential centrifugation, and the different fractions were separated as follows: structural proteins, nucleus, and cell debris at 600×g for 10 min; mitochondria at 5000×g for 10 min; lysosomes at 15,000×g for 10 min. Myocardial subfractions were treated with Triton X-100 (final concentration 0.2% *v*/v) in ice for 15 min prior to the determination of enzymatic activity (Venkatachalem et al. 2003).

The activities of the lysosomal enzymes like β-glucuronidase (Hultberg et al. 1976), β-glucosidase and β-galactosidase (Conchie et al. 1967), and acid phosphatase (Kavanagh and Bardsley 1979) were determined.

Determination of tissue antioxidants

At the end of the experimentation, hearts were excised from rats and homogenized in 0.1 M Tris buffer (pH 7.4), obtained homogenate was centrifuged at a speed of 2500 rpm. The attained supernatant was used for estimation of tissue antioxidants like super oxide dismutase (SOD) (Misra and Fredovich., 1972), Reduced glutathione (GSH) (Moran S. et al. 1979), Catalase (Aebi. H., 1984) and lipid peroxidation (LPO) (Kovachich and Mishra. 1980).

Histopathological studies of heart

After removal of myocardial tissue immediately washed with ice cold saline to remove all the blood and fixed in 10% buffered neutral formalin solution. After fixation was complete, tissues were embedded in paraffin and serial sections were cut in to 0.5 μm. Each section was stained with hematoxylin and eosin. The sections were examined under light microscope and histograms were taken.

Statistical analysis

Results were expressed as mean ± standard error mean multiple comparisons of the significant analysis of variance (ANOVA) followed by the Dennett's test as post parametric test using computer based fitting program (Prism graph pad 5.0). A p value of <0.05 was considered as statistically significant.

Results

The preliminary phytochemical evaluation of ethanolic extract *Rosa damascena* Mill. L. (Table 1) reveals the presence of plant secondary metabolite like cardiac glycosides, tannins, flavonoids, polyphenols and carbohydrates. These are the secondary metabolites of the plant with various biological activities.

The results of acute toxicity studies have revealed that there was no toxicity or lethality found at the dose of 2000 mg/kg body weight. For the assessment of cardioprotective activity, dose levels were chosen in such a way that, doses were approximately one tenth (200 mg/kg) and one fifth (400 mg/kg)

Table 1 Preliminary phytochemical evaluation of ethanolic extract of *Rosa damascena* Mill. L.

Name of the test	Result
Flavonoids	++
Phenols	++
Alkaloids	++
Saponins	++
Carbohydrates	++
Proteins & Amino acids	++
Tannins	++
Cardiac glycosides	++

of the maximum dose employed in acute toxicity studies i.e. 2000 mg/kg body weight.

The changes in serum cardiac biomarkers like CK-MB and LDH were tabulated in Table 2. The concentration of CK-MB and LDH were significantly ($p < 0.05$) increased in isoproterenol alone treated rats compared to normal control rats. Pre-treatment with ERD significantly ($p < 0.05$) attenuates these markers levels when compared with disease control rats.

The effect of ERD on membrane bound Na^+/K^+ ATPase activity has shown in Table 2, significant ($p < 0.05$) decrease of myocardial membrane bound Na^+/K^+ ATPase in rats treated with ISO alone when compared with normal rats. Whereas the same Na^+/K^+ ATPase significantly ($p < 0.05$) increased in rats pretreated with ERD for four weeks when compared with disease control rats.

The membrane stabilizing activity has shown in (Fig. 1). ERD treatment exhibited at all the concentration has significantly ($p < 0.05$) protected the red blood cell membrane damage against heat and hypotonic saline induced hemolysis when compared with control. The increased concentration of plant extract increases the activity of membrane stabilization. The maximum membrane stabilization activity was observed at highest concentration (200 μg/ml).

Table 3 shows that alterations of activities of lysosomal hydrolases enzymes like β-glucuronidase, β- galactosidase, β-glucosidase, and acid phosphatase. These activity levels were significantly ($p < 0.05$) increased in heart tissue homogenates of rats treated with isoproterenol alone when compared with normal rats. Animals pre-treated with ethanolic extract of *Rosa damascene* mill. L. at doses of 200 and 400 mg/kg brought these enzyme activities significantly ($p < 0.05$) low near to normalcy when compared with animals treated with isoproterenol alone treated rats.

The changes in heart antioxidants are presented in Table 4. In ISO induced rats, there was a significant ($p < 0.05$) decrease in GSH, Catalase, SOD and a significant increase in LPO compared to normal control group and pre-treatment with ERD at doses of 200 and 400 mg/kg, respectively, brought the elevated heart antioxidants near to normalcy.

Histopathological observations (Fig. 2) of normal control rat's heart revealed normal cardiac muscle bundles without any inflammation. Whereas rat's myocardium treated with isoproterenol alone has shown marked inflammatory signs like membrane damage and cellular infiltration along with focal myonecrosis. Rats pre-treated with ERD at 200 mg/kg and 400 mg/kg has shown reduction in inflammatory signs and myonecrosis compared to disease control.

Discussion

The phytochemical analysis of present study reveals the presence of polyphenols, flavonoids and cardiac glycosides. It has been reported that upon the administration of isoproterenol, myocardial cells were damaged or destroyed due to the deficiency of oxygen supply or glucose and production of more lactate, the cell membrane becomes permeable or may rupture and results in the leakage of enzymes like CK, LDH, and transaminases in serum with a subsequent decrease in the myocardium (Rajadurai and Prince 2007). The levels of these enzymes present in plasma or serum are reported to be directly proportional to the number of necrotic cells present in the cardiac tissue (Geetha et al. 1990). The increased levels of these enzymes is due to their leakage from cardiac cells as an outcome of lipid peroxidation induced by ISO which has ultimately lead to membrane damage and altered permeability. Present investigations found that there is marked elevation of serum cardiac markers (CK-MB and LDH) in isoproterenol

Table 2 Effect of ERD on serum CK-MB, LDH levels and Na^+/K^+ ATPase activity

Treatment	CK-MB (IU/L)	LDH (IU/L)	Na^+/K^+ ATPase activity (μmol of Pi/mg protein/h)
Normal control (normal saline)	83.89 ± 0.363	164.8 ± 0.44	3.865 ± 0.109
Ischemic control (ISO 100 mg/kg)	347.8 ± 0.496[a]	422.2 ± 0.352[a]	1.859 ± 0.487 [a]
Standard (Metaprolol10mg/kg)	98.98 ± 0.32	251.6 ± 1.89	2.916 ± 0.105
ERD (200 mg/kg)	153.9 ± 0.36[b]	316.8 ± 0.217[b]	2.087 ± 0.116[b]
ERD (400 mg/kg)	136.5 ± 0.43[b]	285.5 ± 0.430 [b]	2.677 ± 0.107 [b]

All Results are the mean ± SEM for six animals; one-way ANOVA; Dennett's multiple comparison tests. a = $p < 0.05$, when compared with normal group. b = $p < 0.05$, when compared with ischemic control group

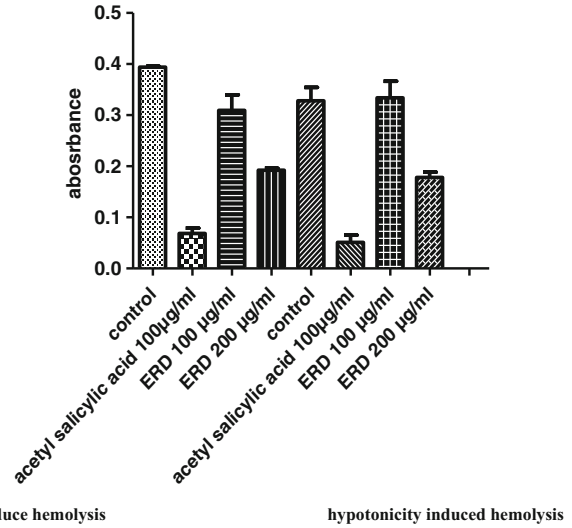


Fig. 1 Effect of ERD on heat and hypotonicity induced hemolysis

alone treated rats, whereas the same was effectively decreased in rats pre-treated with ERD (Table 2). This indicates that the *Rosa damascena* has capability to protect myocardium from free radical stress.

Na^+- K^+ ATPase is rich in thiol groups, oxidation of thiol groups by reactive oxygen species is reported to inhibit enzyme activity (Thomas and Reed 1990). The auto-oxidation of administered catecholamines liberates free radicals which ultimately bind to the thiol group of the enzyme and thereby inhibit its function. It was distinguished that Na^+ /K^+ ATPase activity was significantly inhibited in ISO-induced rats when compared to normal control. Rats pre-treatment with ERD at doses of 200 and 400 mg/kg significantly prevented the decrease of the levels of Na^+ /K^+ ATPase activity when compared to diseased control group. This effect may be by cell viability and membrane stabilization.

Membrane stabilization leads to the prevention of leakage of serum proteins and fluids into the tissues during a period of increased permeability caused by inflammatory mediators. The red blood cells upon exposure to hypotonic solution and heat, it results in damage of the membranes, followed by hemolysis and oxidation of hemoglobin. ERD at all the concentrations has exhibited the red blood cell membrane stabilization and is dose dependent. Since red blood cell's membrane is similar to the lysosomal membrane components, the prevention of hypotonicity-induced cell lysis was taken as measure of anti-inflammatory activity of drugs (Chioma et al. 2012). Inflammatory reaction is the main pathological process in the early period of MI (Chen Huaguo et al. 2015). During isoproterenol induced myocardial infarction a prominent inflammation occurs in myocardium. The membrane stabilization action of ERD probably reduces the myocardial inflammation to contribute its protection in ISO induced MI.

Damage to lysosomal membrane causes elevation of hydrolyzing enzymes in both intra and extra-cellular space, which lead to cellular and tissue necrosis including apoptosis (George 2008). Lysosomal hydrolases play an important role in ISO induced MI which is mediated by pro-inflammatory mediators (Prabhu et al. 2009). ISO treatment produces quinines, which react with oxygen to produce superoxide anion and hydrogen peroxide, leading to oxidative stress thereby damaging the myocardial cells. It was observed that the increased levels of lysosomal lipid peroxidation in myocardial infarcted rats are the reason for the leakage of myocardial lysosomal enzymes from the lysosomes due to their membrane damage by isoproterenol (Subhro and Ponnian 2012).

The present study witnessed that ISO treated rats shows decreased membrane stabilizing activity which ultimately lead to increases in the liberation of lysosomal hydrolyzing enzymes like β-glucuronidase, β- galactosidase, β-glucosidase, and acid phosphatase from their sacs in to the myocardial cells and causes infarction. Pre-treatment with ERD in ISO infarcted rats could effectively inhibit the release of these enzymes from their sacs, this effect could be due to its membrane stabilizing action on lysosomal membrane. The current study is

Table 3 Effect of ERD on lysosomal enzymes of heart homogenates

Treatment	β-Glucuronidase (μ moles of p-nitrophenol /h/100 mg of protein)	β-Glucosidase (μ moles of p-nitrophenol /h/100 mg of protein)	β-Galactosidase (μ moles of p-nitrophenol /h/100 mg of protein)	Acid phosphatase (μ moles of phenol /mg of protein)
Normal control (normal saline)	16.15 ± 2.14	11.35 ± 2.0 1	32.53 ± 5.81	46.59 ± 2.47
Ischemic control (ISO 100 mg/kg)	82.20 ± 1.22[a]	65.24 ± 1.13 [a]	85.50 ± 3.15 [a]	97.68 ± 4.33 [a]
Standard (Metaprolol10mg/kg)	52.72 ± 2.49	45.53 ± 5.81	44.68 ± 4.65	56.95 ± 1.42
ERD (200 mg/kg)	43.58 ± 1.82 [b]	48.28 ± 2.47 [b]	59.62 ± 5.34 [b]	69.65 ± 1.63 [b]
ERD (400 mg/kg)	42.67 ± 1.82 [b]	45.81 ± 2.47 [b]	56.46 ± 2.34 [b]	61.65 ± 3.23 [b]

All Results are the mean ± SEM for six animals; one-way ANOVA; Dennett's multiple comparison tests. a = $p < 0.05$, when compared with normal group. b = $p < 0.05$, when compared with ischemic control group

Table 4 Effect of ERD on tissue antioxidants in heart homogenate

TREATMENT	SOD (U/mg Protein)	CATALASE (μM of H2O2Consumed/mg Protein)	GSH (μg of GSH/ mg Protein)	LPO (nM of MDA/ mg Protein)
Normal control (normal saline)	8.422 ± 1.215	2.300 ± 0.669	47.94 ± 1.36	1.579 ± 0.116
Ischemic control (ISO 100 mg/kg)	1.602 ± 0.087[a]	0.9550 ± 0.160[a]	8.602 ± 0.349[a]	3.213 ± 0.413[a]
Standard (Metaprolol10mg/kg)	7.801 ± 0.933	1.927 ± 0.183	42.21 ± 2.37	1.773 ± 0.130
ERD (200 mg/kg)	5.868 ± 0.541[b]	1.518 ± 0.085[b]	35.42 ± 1.70[b]	2.036 ± 0.131[b]
ERD (400 mg/kg)	6.427 ± 0.776[b]	1.660 ± 0.100[b]	37.88 ± 2.10[b]	1.970 ± 0.122[b]

All Results are the mean ± SEM for six animals; one-way ANOVA; Dennett's multiple comparison tests. a = $p < 0.05$, when compared with normal group. b = $p < 0.05$, when compared with ischemic control group

in accordance with Seyedeh and his co-workers stated that high phenolic content of extract of *R. damascena* could be responsible for its high antioxidant activity (Seyedeh and Iraj 2010) and also reports from Ali and his co-workers indicated that *R. damascena* could have a potential reducing effect in the production of free radicals (Ali et al. 2011). Considering the above statements may accomplish that the *R. damascena* could have the capability to prevent free radical induced lysosomal and myocardial membrane destabilization during MI.

Quinines produced by the ISO may react with oxygen to produce superoxide anion and hydrogen peroxide, leading to oxidative stress thus damages the myocardial cells. The excessive formation of free radicals as well as accumulation of lipid peroxides has been predictable for the possible mechanisms for the myocardial damage caused by ISO (Sathish et al. 2003). However, the decreased activities of antioxidant enzymes SOD, GSH and Catalase in the heart may be due to the ISO-induced reactive oxygen species, such as superoxide and hydrogen peroxide. *Rosa damascena* with its potent antioxidant effect may inhibit the effect of reactive oxygen species generated by the isoproterenol, and responsible for increasing the activity of heart antioxidative enzymes in ERD pretreated rats.

Biochemical findings that proves the cardioprotecctive effect was further supported by histopathological examination of heart. Myocardial tissue sections of normal rats depicted clear integrity of the myocardial cell membrane whereas the sections of hearts treated with ISO showed necrosis of muscle fibers with inflammatory cell infiltration, edema and fragmentation of muscle fibers, which indicated involvement of oxidative stress and inflammatory processes. Pre-treatment with ERD showed the integrity to the structure of myocardium from ISO injury near to the normal myocardium denotes its cardio protective action.

The existed pharmacological data on the *Rosa damascena* reveals that the components of the plant such as terpenes,

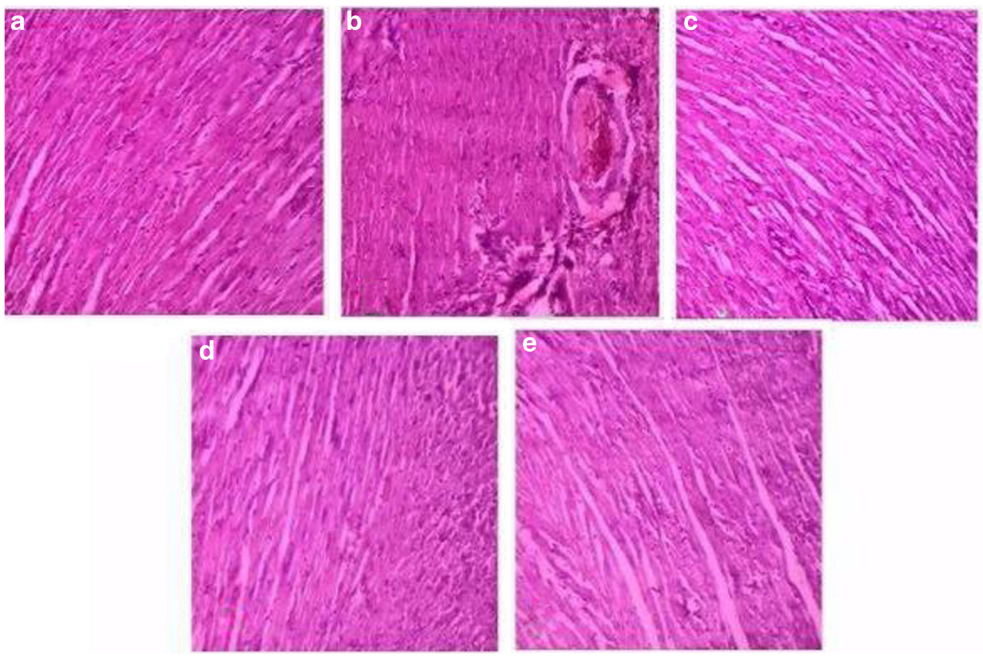


Fig. 2 **a**–**e**: Histopathological study of the heart. **a** Normal control rat's heart showing normal texture of cardiac muscle bundles. **b** Disease control treated with isoproterenol induced MI rat's heart showing area of infarction with inflammatory cell infiltration, cardiac necrosis and splitting of muscle bundles. **c** Metoprolol treatment rat's heart showing maintains the myocardium from ISO injury mild inflammatory infiltrate without any infarction. **d**, **e** ERD pre-treatment rat's heart showing the prevention of cell infiltration, splitting of muscle bundle by ISO has been prevented

glycosides, flavonoids, and anthocyanins have capacity to encounter various diseases of humans as antidiabetic, antimicrobial, anti-HIV, anti-inflammatory, and antioxidant. Here the present study emphasis for *Rosa damascena*'s protection against ISO induced cardio toxicity by stabilizing the myocardial and lysosomal membrane, which has not been appraised so far.

Conclusion

The present study concludes that *Rosa damascena* Mill. L. could effectively reduce myocardial damage and attenuates isoproterenol-induced lysosomal membrane destabilization by preventing the leakage of its enzyme. Understanding the mechanisms involved in the lysosomal enzyme leakage may prove beneficial measures in the prevention of myocardial infarction. Thus, lysosomal stabilization treatment may be effective in the prevention of myocardial infarction. Further studies are needed to isolate the chemical constituents and establishment of their mechanism of action in the protection of myocardial infarction.

Acknowledgements Authors are thankful to principal and management of Sri Padmavathi School of Pharmacy for providing the laboratory facilities and ethical committee approval.

Compliance with Ethical Standards

Ethical Statement All the experimental protocols were described and approved by the Institutional Animal Ethical Committee of Sri Padmavathi School of Pharmacy, Tirupati.

Conflict of Interest All the authors declare that they have no conflicts of interest.

References

Aebi H (1984) In: Catalase in Vitro. Methods in Enzymology. Colowick SP, Kaplan NO, editors. Vol. 105. Florida: Acad. Press; pp. 114–121

Ahmed AZ, Nadia EH, Mohamed A, Mohamed FL (2014) Cardioprotecctive and antioxidant effects of oleogum resin Olibanum from Boswellia carteri Birdw. (Bursearceae). Chin J Nat Med 12:0345–0350

Ali DK, Iraj R, Mohammad BR, Parviz O (2011) Antioxidative properties and toxicity of white rose extract. Iran J Toxicol 5:415–425

Chioma AA, Obidoa O, Lawrence US (2012) Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). DARU J Pharm Sci 20:1–7

Conchie J, Gelman AL, Levvy GA (1967) Inhibition of glycosidases by aldonolactones of corresponding configuration. The C-4 and C-6 specificity of beta glucosidase and beta galactosidase. Biochem J 103:609–615

Daniela G, Dietmar E, Heribert S, Michael P, Daniel G, Gunter B, Frank M, Gunter AJ, Eckhard PK (1998) Development of heart failure following isoproterenol administration in the rat: role of the renin-angiotensin system. Cardiovasc Res 37:91–100

Gadham SK, Mohammed AK, Vaddi DR, Pannuru P, Shaik Althaf H, Lakshmi DK (2012) *Aegle marmelos* fruit extract attenuates isoproterenol-induced oxidative stress in rats. J Clin Biochem Nutr 50:199–204

Geetha A, Sankar R, Marar T et al (1990) Alpha-tocopherol reduces doxorubicin-induced toxicity in rats–histological and biochemical evidences. Indian J Physiol Pharmacol 34:94–100

George J (2008) Elevated serum beta-glucuronidase reflects hepatic lysosomal fragility following toxic liver injury in rats. Biochem Cell Biol 86:235–243

Huaguo C, Xu Y, Wang J, Wei Z, Huihui R (2015) Baicalin ameliorates isoproterenol-induced acute myocardial infarction through iNOS, inflammation and oxidative stress in rat. Int J Cli Exp Pathol 8: 10139–10147

Hultberg B, Lindsten J, Sjoblad S (1976) Molecular forms and activities of glycosidase in cultures of amniotic-fluid cells. Biochem J 155: 599–605

Kalra J, Prasad K (1994) Oxygen free radicals and cardiac depression. Clin Biochem 27:163–168

Karthikeyan BR, SaralaBai S, Niranjali D (2007) Grape seed proanthocyanidins ameliorates isoproterenol-induced myocardial injury in rats by stabilizing mitochondrial and lysosomal enzymes: an *in vivo* study. Life Sci 81:1615–1621

Kavanagh JP, Bardsley WG (1979) The identity of the acid and alkaline phosphatases of human seminal plasma. J Reprod Fertil 57:43–48

Khandelwal KR (2002) Practical pharmacognosy techniques & experiments, 9th ed, Nirali Prakashan, pp 156–161

Kovachich GB, Mishra OP (1980) Lipid peroxidation in rat brain cortical slices as measured by the thiobarbituric acid test. J Neurochem 35: 1449–52

Misra HP, Fridovich I (1972) The role of super oxide anion in autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 247:3171–3175

Mohammad HB, Mohammad NS, Zahra SS (2001) Pharmacological effects of Rosa damascena. Iran J Basic Med Sci 4:295–307

Moran M, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione s transferase activities in rat lung and liver. Biochem Boiphys Acta. 582:67–68

Periyathambi TD, Ponnian SM (2007) (−)-Epigallocatechin gallate (EGCG) prevents isoprenaline-induced cardiac toxicity by stabilizing cardiac marker enzymes and membrane-bound ATPases. J Pharm Pharmacol 60:125–133

Prabhu S, Shoba N, Shyamala D (2009) Mechanism of protective action of Mangiferin on suppression of inflammatory response and Lysosomal instability in rat model of myocardial infarction. Phytother Res 23:756–760

Rajadurai M, Prince SM (2007) Preventive effect of naringin on Isoproterenol-induced cardio toxicity in Wistar rats: an in vivo and in vitro study. Toxicology 232: 216-25

Rona G, Chappel CI, Balazs T, Gaudry R (1959) An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. A.M.A .Arch Pathol 67:443–455

Sathish V, Ebenezar KK, Devaki T (2003) Synergistic effect of nicorandil and amlodipine on lysosomal hydrolases during experimental myocardial infarction in rats. Biomed Pharmacother 57:309–313

Seyedeh MS, Iraj R (2010) Cytobiochemical potentials of *Rosa damascena* Mill. extract. Iran J Pathol 5:184–193

Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN (1999) Membrane stabilizing activity-a possible mechanism of action for the anti inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 70:251–257

Subhro JR, Ponnian SMP (2012) Protective effects of sinapic acid on lysosomal dysfunction in isoproterenol induced myocardial infracted rats. Food Chem Toxicol 50:3984–3989

Thomas CE, Reed D (1990) Radical-induced inactivation of kidney Na^+/K^+ ATPase: sensitivity to membrane lipid peroxidation and the protective effect of vitamin E. Arch Biochem Biophys 281:96–105

Venkatachalem S, Kesavarao KE, Thiruvengadam D (2003) Synergistic effect of nicorandil and amlodipine on lysosomal hydrolases during experimental myocardial infarction in rats. Biomed Pharmacother 57:309–313