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Research Article

Antioxidant Activity of *Rosa damascena* in Isoproterenol Induced in Oxidative Stress in Rats

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Abstract

Anti-oxidant activity of ethanolic extract of *Rosa damascena* (200 and 400 mg/kg b.wt, orally for 28 days) against isoproterenol-induced oxidative stress was investigated in rats. In addition to the activities of the antioxidant enzymes-glutathione reductase, superoxide dismutase, catalase and lipid peroxidase in the heart tissue. Administration of isoproterenol increased the level of lipid peroxides with corresponding decrease in the activities of the non-enzymatic antioxidants. The pre-treatment with ethanolic extract of petals significantly prevented the alterations induced by isoproterenol, and maintained near normal antioxidant status. Results suggest that the cardioprotective effect of *Rosa damascena* may partly be attributed to its antioxidant properties.

Keywords: *Myocardial Infraction; Cardio Protection; Biomarkers; Antioxidants*

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. Myocardial infection (MI) results from any interruption in the blood supply to heart, at leads to death of the cardiac tissue, myocardial necrosis. The consequences of MI include hyperlipidemia, peroxidation of membrane lipids, and loss of plasma membrane integrity. Isoproterenol- induced myocardial ischemia is considered as one of the most widely used experimental models to study the beneficial effects of many drugs and cardiac functions. The highly reactive cytotoxic free radicals through auto-oxidation of catecholamine have been involved as one of the important causative factors for isoproterenol (ISO) induced cardiac damage.

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 rosa 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, inotropic agent, for the treatment of menstrual bleeding, antitussive, tracheal relaxant, and relaxing activity are the other effects that attributed to *Rosa damascena* Mill *L.* [1].

The major background of the present study is, administration of isoproterenol to liberate oxygen free radicals to lead to damage or destabilization. The present study was designed to variety the hypothesis, that oxygen deficiency followed by free radical generation by ISO. The investigated the defensive effect of *Rosa damascena* Mill L. extract on the above activity against ISO induced myocardial infraction in rats.

Materials and Methods

Collection of plant material

The flower 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. B. Sitaram, Professor, Department of Dravyaguna, S.V. Ayurvedic Medical College, Tirupati.

Preparation of plant extract

The fresh petals of flower *Rosa damascene* were shade dried. The dried petals were grinded to get coarse powder. 250 gm of coarse powder was subjected to cold maceration process using hydroalcohol (70:30) as solvent. The extraction was continued for 3 days at room temperature with occasional shaking. Then the extract was filtered, collected and concentrated at 70°C on a heating mantle until a softy

mass obtained. It was then thoroughly air dried to remove all the traces of solvent and then was subjected to freeze drying. The obtained plant extract was preserved in cold condition i.e. below 4°C till the end of treatment period.

The preliminary phytochemical screening of ethanolic extract of *Rosa damascena* Mill L. was carried out according to the methods described by Khandelwas., *et al* [2].

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

Healthy Male Wistar strain of about 200 - 250 gm used in this study. Rats were housed under standard conditions and fed with standard pellet with drinking water ad libitum. The animals were caged individually and kept in air conditioned room at temperature of 22 ± 2°C with 50% ± 10% relative humidity with 12hrs light and dark cycle. Throughout the study animals were maintained at normal laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee of Sri Padmavathi School of pharmacy, Tiruchanoor, Tirupati (No: SPSP/CPCSEA/IAEC-1016/a /2014/008). They were purchased from Raghavendra enterprises; Bangalore.

Acute toxicity studies

An acute toxicity study was performed as per Organization for Economic Co-Operation and Development (OECD) 423 guidelines. Single doses 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 infraction

Myocardial infraction 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 [3].

Experimental schedule

The treatment schedule was fixed for 28 days. 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 (85 mg/kg body weights) 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, group IV and V received 200 and 400 mg/kg body weight of extract of *Rosa damascena*, 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.

Determination of tissue antioxidants

At the end of the experimentation, heart 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 tissues antioxidants like super oxide dismutase (SOD) [4], Reduced glutathione (GSH) [5], Catalase [6] and lipid peroxidation (LPO) [7].

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 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 activates.

S. No	Name of the Test	Result
1	Flavonoids	++
2	Phenols	++
3	Alkaloids	++
4	Saponins	++
5	Carbohydrates	++
6	Proteins and amino acids	++
7	Tannins	++
8	Cardiac glycosides	++

Table 1: *Results of Preliminary Phytochemical Screening of ERD.*

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 200 mg/kg and 400 mg/kg of the maximum dose employed in acute toxicity studies i.e. 2000 mg/kg body weight.

Effect of ERD on *in vivo* antioxidant GSH levels of heart homogenates

There was significant ($p < 0.05$) decrease of *in vivo* antioxidant of GSH levels in ischemic control when compared with normal control. There was significant ($p < 0.05$) increase in *in vivo* antioxidant of GSH levels in groups treated with test drug at doses of 200 and 400 mg/kg, p.o, when compared with ischemic control group.

Effect of ERD on *in vivo* antioxidant SOD levels of heart homogenates

There was significant ($p < 0.05$) decrease of *in vivo* antioxidant of SOD levels in ischemic control when compared with normal control. There was significant ($p < 0.05$) increase in *in vivo* antioxidant of SOD levels in groups treated with test drug at doses of 200 and 400 mg/kg, p.o, when compared with ischemic control group.

Effect of ERD on *in vivo* antioxidant CATALASE levels of heart homogenates

There was significant ($p < 0.05$) decrease of *in vivo* antioxidant of catalase levels in ischemic control when compared with normal control. There was significant ($p < 0.05$) increase in *in vivo* antioxidant of catalase levels in groups treated with test drug at doses of 200 and 400 mg/kg, p.o, when compared with ischemic control group.

Effect of ERD on *in vivo* antioxidant Lipid peroxidation levels of heart homogenates

Lipid peroxidation levels were significantly ($p < 0.05$) increased in ischemic control group when compared with normal control. Lipid peroxidation levels were significantly ($p < 0.05$) decreased in low dose and high dose treated groups when compared with ischemic control group.

S. No	Treatment	SOD (U/mg Protein)	Catalase (μMH_2O_2Consumed/mg protein)	GSH (µgof GSH/mg Protein)	LPO (nMof MDA/mg Protein)
1	Normal saline	8.422 ± 1.215	2.300 ± 0.669	47.94 ± 1.36	1.579 ± 0.116
2	Ischemic control	1.602 ± 0.087[a]	0.9550 ± 0.160[a]	8.602 ± 0.349[a]	3.213 ± 0.413[a]
3	Standard	7.801 ± 0.933	1.927 ± 0.183	42.21 ± 2.37	1.773 ± 0.130
4	Test (200 mg/kg)	5.868 ± 0.541[b]	1.518 ± 0.085[b]	35.42 ± 1.70[b]	2.036 ± 0.131[b]
5	Test (400 mg/kg)	6.427 ± 0.776[b]	1.660 ± 0.100[b]	37.88 ± 2.10[b]	1.970 ± 0.122[b]

Table 2: *Effect of ERD on Antioxidant enzymes in heart homogenate.*

Discussion

There is a huge burden of cardiovascular disease as it is one of the foremost health problem globally and reaching wide spread extents in the India. It is standing first among top 5 causes of deaths in Indian population. Myocardial infarction (MI) ensues as a consequence of sustained myocardial ischemia that precipitates irreparable injury and necrosis of tissue of myocardium due to insufficient supply of blood. In most of the people due to atheromatous plaque disruption and thrombus formation within the coronary circulation MI occurs [8].

The current investigation is intended to assess and discover the cardioprotective effect of *Rosa damascena* oxidative stress induced by isoproterenol in rats. Soni Himesh., *et al.* elucidated that therapeutic worth of the extract may be due to its antioxidant, lipid peroxidative resistant and free radical scavenging activity [9].

The phytochemical screening outcomes of these flowers by Ram Swaroop Verma., *et al.* revealed the presence of phytoconstituents such as quercetin (pentahydroxyflavone), kaempferol (tetrahydroxyflavone), rutin, myricetin, apigenin, catechin, epicatechin, cyanidin 3,5-diglucoside, phenolic acids, majors carotenoids like b-carotene, lycopene, rubixanthin, zeaxanthin, lutein and vitamin C [10]. Parul Lakhanpal., *et al.* evidently illuminated the role of quercetin, bioflavonoids, vitamin C in reducing risk of cardiovascular diseases. Mukesh Nandave., *et al.* also concluded that flavonoids are considered to be potent antioxidants and can be proved as cardioprotective by prevention and delay of destructive oxidative reactions in cells which may influence the progression of cardiovascular diseases [11].

Radical scavenging activities and natural indicator activity of aqueous and ethanolic extract of Rosa damascena was done by Soni Himesh., et al. and witnessed that due to the presence of the above mentioned phytoconstituents these flowers are exhibiting free radical scavenging activity [9]. Due to the defensive effect of the *Rosa damascena* on the myocardium, reduced extent of structural and functional myocardial damage and thereby prevention of the leakage of diagnostic cardiac enzymes from the myocardium.

Isoproterenol (ISO) is a conventional cardiotoxic agent for its capability to damage cell of myocardium. It is a powerful synthetic catecholamine which produces infarct like abrasions or lesions in animals when injected and these abrasions are similar morphologically to degeneration myofibrillar tissue which is observed during acute MI and unexpected death in man (Rathore., *et al*. 1998). There exist numerous investigation which suggest that administration of catecholamines in huge doses produces necrosis in myocardium. The reason for this is due to increase in cAMP levels, overload of intracellular calcium and high energy phosphates lassitude. On administration of isoproterenol noteworthy decrease seen in cardiac marker enzymes activities and ultimately leads to ensuing upsurge in the activities of cardiac enzymes in the serum [12]. Murugesan., *et al.* in their work noticed that the liberation of cardiac enzymes echoes the variations in integrity plasma membrane and permeability [13].

The auto-oxidation of catecholamines cause production highly cytotoxic free radicals and the localization of highly unsaturated fatty acids in membrane make them susceptible to lipid peroxidation. Due to β-adrenergic action by ISO administration leads to reduction of GSH level along with antioxidant enzymes like SOD and Catalase in myocardium tissue [13]. M Nagasaraswathi., *et al*. found the reason for decrease in the activity of Glutathione Peroxidase (GPx) may be related to decrease accessibility of its substrate that is reduced GSH. Due to the damage of both enzymatic defense mechanism and non-enzymatic defense mechanism of antioxidants the free radicals will not be completely neutralized and so the myocardium shows boosted proneness to lipid peroxidation [14].

Soni Himesh., *et al*. worked on *Rosa damascena* flowers and found out the therapeutic worth of the flower as potent antioxidant, lipid peroxidative resistant and free radical scavenging activity as due to the presence of magnificent amount flavonoids [9].

Pre-treatment with ERD (200 mg/kg and 400 mg/kg., p.o) and then treated with ISO (85 mg/kg) revealed the significant increase in the level of antioxidant activity of SOD and catalase, so it is proving that ERD can efficiently increases SOD which can scavenge the free radical superoxide prior formed in the system and catalase can remove hydrogen peroxide to protect the myocardium from harmful effects.

Pretreatment with ERD (200 mg/kg and 400 mg/kg., p.o) prevented the ISO- induced lipid peroxidation and maintained the level of reduced glutathione near to normal level in heart. This might be due to antioxidant activity of *Rosa damascena*.

GSH and Catalase protect SOD from being inactivated by hydrogen peroxide (H_2O_2). In return SOD safeguards Catalase and GSH from being inhibited by radicals of superoxide [15]. Hence the equilibrium between these enzyme systems is vital to maintain the stable state levels of the oxygen radicals low. The test drug contains quercetin and kaempferol which can restore normal activities of these enzymes suggesting that the flowers could considerably improve cellular antioxidative defense against oxidative stress.

LPO is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme inactivation. It is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane. Free radical facilitated impairment mainly occurs due to lipid peroxidation along with thiol group's reduction (Singal., *et al*. 1983). Lipid peroxidation is considered to be an indication of severe condition of ISO induced necrotic damage of heart and also associated with changed structure of membrane and inactivation of enzymes [16]. Major lipid peroxidation end product is malondialdehyde. Increase in content of Malondialdehyde adds to increase in production of free radicals and decrease in activities of antioxidant system [17]. The previous studies have specified that ISO induced MI could be due to the generation of free radical facilitated lipid peroxidation as an outcome of condition of stress in rats.

In ISO treated rats elevation malondialdehyde content was observed it might be due to the generation of free radical facilitated lipid peroxidation as an outcome of condition of stress in rats. The results presented in this study indicated that the pretreatment with *Rosa damascena* extract could decrease ISO induced malondialdehyde content elevation. The decreased level of malondialdehyde in heart tissues might be due to enhanced activities in antioxidant enzymes (superoxide dismutase, catalase, GSH). This proves the effective neutralization and scavenging property which resulted in cardioprotective effect of *Rosa damascena*.

The existed pharmacological data on the *Rosa damascena* reveals that the components of the plant such as terpenes, glycosides, flavonoids, and anthocyanins have capacity to encounter various diseases of human as Antidiabetic, antimicrobial, anti-HIV, anti-inflammatory, and antioxidant. Here the present study emphasis for *Rosa damascene's* protection against ISO induced cardio toxicity by stabilizing the myocardial infraction [18,19].

Conclusion

The present study concludes that *Rosa damascena* Mill *L.* Could effectively reduce myocardial damage from the result it can conclude that treatment with ERD at various doses as 200 mg/kg and 400 mg/kg has shown a significant cardio protective activity in dose dependent manner. Further studies on Rosa damascene can be extended by the isolation of individual chemical component which are responsible for mechanism of action of cardio protection against Isoproterenol induced oxidative stress. Further studies are needed to isolate the chemical constituents and establishment of their mechanism of action in the protection of myocardial infraction. The present study attempts to evaluate the effect ERD on Isoproterenol induced MI. At the end of the study all the treatment groups were treated with isoproterenol 85 mg/kg for two consecutive days to induce oxidative stress.

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