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Antioxidant effects of a *Rhodobryum roseum* extract and its active components in isoproterenol-induced myocardial injury in rats and cardiac myocytes against oxidative stress-triggered damage

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The aim of this study was to investigate (1) whether *Rhodobryum roseum*, a traditional Chinese medicine used to treat cardiac disease, can protect myocardium damage due to isoproterenol-induced injury, (2) whether the cardioprotective effect of the *R. roseum* extract is related to its antioxidant activity, and (3) to identify the active components of *R. roseum* using the oxidant-mediated injury in cardiomyocytes. *R. roseum* was extracted with 95% EtOH (RE-95), 50% EtOH (RE-50) and water (Re-H_2O) and the rats were treated orally for 11 days at doses of 250 mg and 63 mg/kg respectively after cardiac necrosis was induced by administering ISO subcutaneously at a dose of 85 mg/kg body weight. Levels of marker enzymes (LDH, GOT and CK) were assessed in serum whilst the antioxidant parameters, superoxide dismutase (SOD), and malondialdehde (MDA) were assayed in heart homogenate. Significant myocardial necrosis, depletion of endogenous antioxidants and an increase in serum levels of marker enzymes was observed in ISO-treated animals when compared with the normal animals. The RE-50 elicited a significant cardioprotective effect by lowering the levels of serum marker enzymes, lipid peroxidation (MDA). To extend this work, we sought to investigate the antioxidant effects of the components of *R. roseum*, using the neonatal rat cardiomyocytes model of H_2O_2-induced oxidant injury. Among the four major components, piperine and methyl piperate significantly reduced the medium level of CK and LDH at a variety of dosages. Moreover, piperine and methyl piperate significantly attenuated 2′,7′-dichlorofluorescein (DCF) fluorescence by 63.9% and 52.6%, respectively. The present findings demonstrate that the cardioprotective effects of extracted *R. roseum* in ISO-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation of the membranes. Moreover, its components piperine and methyl piperate exert significant protectective effects on cardiac myocytes.

1. Introduction

Heart ischemia is one of the main cause of sudden death in the world. The pathogenetic mechanism of myocardial ischemic damage is still not completely understood, however, the role of oxygen-derived free radicals in myocardial ischemia (MI) has been well established. The major cytotoxic effect of free radicals in the heart is reported to be the peroxidation of lipid components of cellular and subcellular membranes. This results in the loss of cellular integrity which can lead to irreversible cell injury. Therefore therapeutic interventions with antioxidants or compounds displaying free radical scavenging activity may exert beneficial effects against oxidative stress associated with various cardiovascular diseases, including ischemic heart disease (Bandyopadhyay et al. 2004; Young and Woodside 2001). The enzymes, such as creatine kinase (CK), lactate dehydrogenase (LDH), are increased during myocardial injury, as is the increased level of free radical generating system and malondialdehyde (MDA). Low levels of free radical scavenging systems seem to play a critical role in the instigation of ischemic heart condition (Yogeeta et al. 2006; Pandry et al. 2000).

Recently, several plants of Chinese origin have been found to possess medicinal properties and their beneficial effects in ailments like atherosclerosis, ischemia, cancer, diabetes and liver dysfunction have been attributed to their antioxidant activity. Many traditional plants have been claimed to be useful for the control ischemia and its associated pathologies (Fan et al. 1984).

Rhodobryum roseum Limp. (Chinese name: HuiXinCao) is a medicinal moss used in Yunnan province (China) for the treatment of heart problems. This moss grows throughout Yunnan and is used by many of the ethnic

minority groups in different areas of the province. It has been studied for its pharmaceutical properties in hospitals in Yunnan and elsewhere in China including our laboratory. Additionally, the medicinal uses of *R. roseum* have been documented in popular books on herbal medicine (Eric and Harris et al. 2006) and it has been used in ancient remedys to treat nervous prostration and cardio-vascular diseases in China (Wu 1982; Ding 1982). However, there is little information on its antioxidant activity and its role in cardioprotection (especially on MI); and its major active components are also largely unknown.

Isoproterenol (ISO), a synthetic catecholamine and β adrenergic agonist is documented to produce MI in high doses due to the generation of highly cytotoxic free radicals through its auto-oxidation (Rona et al. 1959). These free radicals stimulate lipid peroxidation and can cause irreversible damage to the myocardial membrane.

Excessive production of H_2O_2 gives rise to events that lead to the death of several types of cells (Wolfe et al. 1994), and in addition, H_2O_2 has been shown to induce apoptosis in cardiomyocytes in culture (Zhang et al. 2001). Furthermore, recent data also show a close link between oxidative stress and apoptosis evoked by ischemia/reperfusion (Khan et al. 2006). It has also been reported that the use of antioxidants can decrease injury and apoptosis through a radical-scavenging mediated mechanism (Dilsiz et al. 2006).

In this study, we report the effects of *R. roseum* extracted compounds on ISO mediated myocardial injury in order to establish its antioxidant activity against myocardium damage and scavenging of hydroxyl radicals. We also used cultured cardiomyocytes which had been exposed to H_2O_2 in order to investigate further the protective effects of the components of *R. roseum.*

2. Investigations and results

2.1. Biochemical parameters

2.1.1. Effects of R. roseum extracts on serum enzyme levels in rats

The effects of *R. roseum* extracts (RE-50, RE-95, RE-H_2O) on serum marker enzymes CK, LDH and GOT are shown in and Table 1. ISO-treated group of animals showed a significant ($p < 0.01$) increase in the activities of marker enzymes CK and LDH as compared to the normal group. RE-50 and RE-95 at doses of 250 mg/kg significantly reduced serum levels CK (to 2056.67 ± 424.23 and 2077.83 ± 630.10 U/L, from 3963.33 ± 554.32 respectively) and LDH (to 537.63 ± 87.67 and 606.58 ± 93.03 U/L, from 801.23 ± 70.28 respectively), whilst RE-50 displayed the most protective effect, however it did not reach the level of the normal control group.

Table 2: Effect of *R. roseum* extracts (RE-50, RE-95 and RE-H_2O) on antioxidant parameters in heart tissue of ISO-induced myocardial injury in rats (mean ± SD)

Groups n=10-12	Malondialdehyde MDA (nmol/g wet tissue)	Superoxide dismutase SOD (U/ mg protein)
Control	200.21 ± 21.35	6.93 ± 0.98
Isoproterenol (85 mg/kg)	$280.25 \pm 25.07^{\#}$	$4.43 \pm 1.50^{\#\#}$
RE-95 (63 mg/kg) + ISO	250.83 ± 37.57	5.47 ± 0.75
RE-95 (250 mg/kg) + ISO	218.33 ± 42.53	$6.95 \pm 1.01^{**}$
RE-50 (63 mg/kg) + ISO	$206.50 \pm 34.89^{*}$	$6.52 \pm 0.99^{*}$
RE-50 (250 mg/kg) + ISO	$205.42 \pm 27.18^{*}$	$6.48 \pm 0.55^{**}$
RE-H_2O (63 mg/kg) + ISO	219.58 ± 23.90	6.05 ± 0.76
RE-H_2O (250 mg/kg) + ISO	250.83 ± 34.08	5.63 ± 1.04

Compared with control: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Compared with isoproterenol: $^{*}P < 0.05$, $^{**}P < 0.01$.

2.1.2. Effects of R. roseum extracts on MDA content and SOD activity of heart in rats

The effects of *R. roseum* extracts (RE-50, RE-95, RE-H_2O) on antioxidant biochemical paradigms are summarized in Table 2. MDA, the myocardial lipid peroxidation marker was significantly elevated ($p < 0.05$) in the ISO control group in comparison with the normal group. Animals treated with RE-50 (250 mg/kg), followed by ISO injection, decreased significantly the increase of MDA (40%, $p < 0.05$), which was approaching values displayed by the normal control group. ISO-induced myocardial necrosis produced a significant depletion in activities of antioxidant enzymes such as SOD ($p < 0.01$) compared to normal animals. However, RE-50 (250 mg/kg and 63 mg/kg) and RE-95 (250 mg/kg) both elevated the myocardial SOD activity.

2.1.3. Effects of active compounds of R. roseum on enzyme levels in cardiomyocyte culture

In H_2O_2-induced injury in neonatal cardiomyocyte, the H_2O_2-treated group of cells showed a significant ($p < 0.01$) increase in the activities of marker enzymes as compared

Table 1: Effect of *R. roseum* extracts (RE-50, RE-95 and RE-H_2O) on serum marker enzymes in ISO-induced myocardial injury in rats (mean ± SD)

Groups n = 10–12	Serum		
	Lactate dehydrogenase GOT(U/L)	Glutamic oxalacetic transaminase LDH(U/L)	Creatine kinase CK(U/L)
Control	157.32 ± 18.78	531.46 ± 89.71	2001.64 ± 500.82
Isoproterenol (85 mg/kg)	$202.32 \pm 13.99^{\#}$	$801.23 \pm 70.29^{\#\#}$	$3963.33 \pm 554.32^{\#\#}$
RE-95 (63 mg/kg) + ISO	170.82 ± 30.94	667.43 ± 125.95	3131.67 ± 513.66
RE-95 (250 mg/kg) + ISO	159.75 ± 19.87	$606.58 \pm 93.03^{*}$	$2077.83 \pm 630.10^{*}$
RE-50 (63 mg/kg) + ISO	169.38 ± 26.43	690.30 ± 98.27	3658.80 ± 696.69
RE-50 (250 mg/kg) + ISO	151.60 ± 15.89	$537.63 \pm 87.67^{**}$	$2056.67 \pm 424.23^{**}$
RE-H_2O (63 mg/kg) + ISO	172.75 ± 27.16	655.87 ± 78.00	3793.00 ± 971.83
RE-H_2O (250 mg/kg) + ISO	156.05 ± 29.23	626.77 ± 85.64	3779.83 ± 476.51

Compared with control: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Compared with isoproterenol: $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 3: Effect of major components of *R. roseum* on LDH and CK in medium of H_2O_2-induced injury neonatal cardiomyocytes

Groups	Dosage	LDH(U/L)	CK(U/L)
Control		52.3 ± 5.5	1.1 ± 0.72
H_2O_2	0.5 mM	$70.8 \pm 8.4^{\#\#}$	$6.6 \pm 0.9^{\#\#}$
	10^{-4} M	71.8 ± 6.5	3.6 ± 0.6
Uridine	10^{-5} M	75.3 ± 2.4	4.6 ± 2.7
	10^{-6} M	71.3 ± 8.8	4.5 ± 2.3
	10^{-4} M	$49.4 \pm 1.4^{**}$	$0.5 \pm 0.4^{**}$
Methyl piperate	10^{-5} M	$52.2 \pm 8.9^{*}$	$0.5 \pm 0.8^{**}$
	10^{-6} M	$50.2 \pm 1.3^{**}$	$1.2 \pm 0.2^{**}$
	10^{-4} M	$47.3 \pm 0.4^{**}$	$1.1 \pm 0.2^{**}$
Piperine	10^{-5} M	$50.5 \pm 9.4^{**}$	$1.3 \pm 1.4^{**}$
	10^{-6} M	$48.2 \pm 1.3^{**}$	$4.2 \pm 1.3^{*}$
	10^{-4} M	66.7 ± 1.4	$3.4 \pm 1.2^{*}$
Caffeic acid methyl ester	10^{-5} M	67.8 ± 2.8	$3.4 \pm 1.3^{*}$
	10^{-6} M	66.2 ± 1.2	$3.2 \pm 1.2^{**}$

Compared with control: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Compared with isoproterenol: $^{*}P < 0.05$, $^{**}P < 0.01$.

to the normal group. Of the test compounds caffeic acid methyl ester reduced the serum levels of CK, while methyl piperate, and piperine significantly reduced serum levels of both CK and LDH at different doses (Table 3). These components did not show any significantly effects on the normal cells (data not shown).

2.1.4. Effects of methyl piperate, and piperine on DCFH oxidation and cell viability in H_2O_2-exposed cardiomyocytes

As shown in the Fig., cells exposed to H_2O_2 (0.5 mM) exhibited a significant increase in DCF fluorescence from 381 ± 15 to 1545 ± 55 a.u. Pretreatment with methyl piperate and piperine (10^{-4} M) attenuated DCF fluorescence in a concentration-dependent manner to 678 ± 56. a.u. ($P < 0.01$), 558 ± 61 a.u. ($P < 0.01$), respectively, compared to H_2O_2 alone. These results were also dose dependent (range from 10^{-6} M to 10^{-4} M).

3. Discussion

R. roseum, a medicinal herb, although not officially listed in the Chinese Pharmacopoeia, has been reported to be

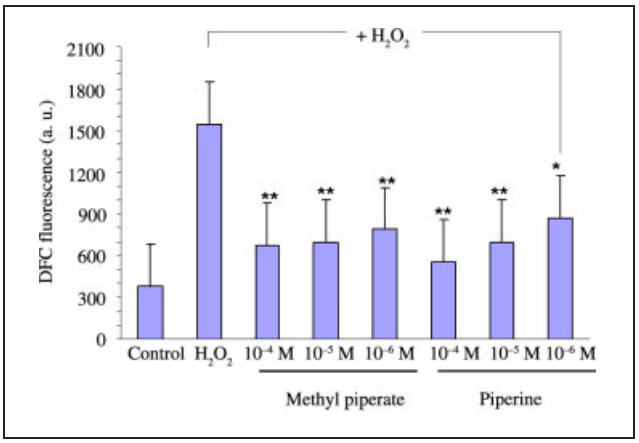
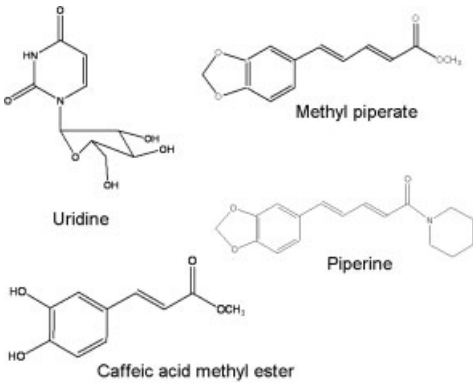


Fig.: Effect of major components from *R. roseum* on DCFH oxidation following the exposure of cardiac cells to H_2O_2.
Cardiomyocytes were loaded with DCFH/DA (10 μM) and exposed to H_2O_2 (0.5 mM) for 2 h or pretreated with methyl piperate, and piperine (10^{-4} M to 10^{-6} M) for 2 h prior to H_2O_2 exposure. $^{*}P < 0.01$, $^{**}P < 0.05$ compared to H_2O_2 alone



effective in treating heart problems in humans. A preparation of *R. roseum* has been demonstrated to alter changes in red cell aggregation and yield-shear stress in dogs with acute experimental coronary occlusion and block hyperviscosity syndrome in acute myocardial ischemia (Yu et al. 1993, 1995). However, there is little information on antioxidant effects of *R. roseum* in myocardial ischemia and of its major active components *in vivo* and *in vitro*. In this study, it is demonstrated that the observed protective effects of extracted of *R. roseum* administration against isoproterenol may have been mediated through endogenous-antioxidant augmenting effects. Moreover, it was shown that piperine and methyl piperate may be the major active components, which can protect the cardiomyocytes from acute exogenous oxidative injury.

Administration of isoproterenol in high concentrations has been reported to induce severe oxidative stress and increases the generation of reactive oxygen species and/or depletes the antioxidants. This may contribute to oxidative stress and affect the pathogenesis of myocardial infarction (Sawyer et al. 2002). Cytosolic enzymes such as lactate dehydrogenase (LDH), glutamic oxalacetic transaminase (GOT) and creatine kinase (CK), which serve as the diagnostic markers of myocardial tissue damage, leak out from the damaged tissues to the blood stream when the cell membrane becomes permeable or ruptured (Sabeena Farvin et al. 2004; Gürgün et al. 2008). The amount of these cellular enzymes presented in plasma reflects the alterations in plasma membrane integrity and/or permeability. Our results show significant elevation in the levels of lactate LDH, GOT and CK in plasma of isoproterenol-treated rats and were indicative of isoproterenol-induced necrotic damage of the myocardial membrane. The prior administration of RE-50 (250 mg/kg) was found to significantly lower the isoproterenol-induced elevation in the activities of diagnostic marker enzymes (CK and LDH).

Moreover, the increased levels of MDA indicate excessive formation of free radicals by auto-oxidation of ISO and activation of the lipid peroxidative process, resulting in irreversible damage to the heart tissue in animals subjected to ISO stress (Zhou et al. 2006; Karthikeyan et al. 2007). RE-50 treatment significantly decreased the MDA levels by preventing formation of lipid peroxides from fatty acids. Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD. This enzyme is a member of mutually supportive enzyme system of the first line cellular, defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more harmful hydroxyl ($OH^{\bullet}$) radical (Lil et al. 1988). In the present study, SOD activity decreased significantly in the ISO

group of animals, probably due to an excessive formation of superoxide anions, which can be harmful to the myocardium (Sharma et al. 2001). Administration of RE-50 to ISO challenged rats effectively prevented the decrease in SOD, which is shown to correlate directly to the scavenging of radicals.

In order to screen the antioxidant activity of the components in *R. roseum*, we investigated which of its components could protect cardiomyocytes from acute oxidant injury using a H_2O_2-induced cardiomyocytes injury model. The test components methyl piperate, and piperine reduced serum levels CK-MB and LDH at different dosages. This could be also due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes. Moreover, when the cells were exposed to exogenous H_2O_2, the DCF fluorescence increased significantly. Since DCF fluorescence represents intracellular oxidative stress (Carter et al. 1994) produced predominantly by the reaction of DCFH-DA with H_2O_2 and hydroxyl radical (Fridovich 1986), this suggests that exogenous H_2O_2 traverses the cell membrane to cause intracellular oxidative stress. Among the four major components, pretreatment with piperine and methyl piperate significantly attenuated the increase in DCF fluorescence caused by exogenous H_2O_2. Our results suggest that piperine and methyl piperate maybe the major active components in *R. roseum* with antioxidant properties and its likely that they are protecting the cardiomyocytes from acute exogenous oxidative injury.

It is concluded that *R. roseum* extracted administration protects rat myocardium damage against isoproterenol-induced myocardial injury against oxidative stress-triggered damage. Because the *R. roseum* extracts used in the present investigation were in a crude form and likely to contain many other components, it is not possible to determine which of these compounds are solely responsible for its protective action. However, its clear that piperine and methyl piperate display antioxidant effects and thus provide protection against free radical induced damage. This supports that notion that herbal medicine exerts their effects through multiple and synergistic mechanisms in the treatment of diseases, due to several active components present in them.

4. Experimental

4.1. Plant material

Rhodobryum roseum (Hedw) Limp was obtained from the Southern part of China (Baoshan District, Yunnan Province), and the pharmacognostic authentication was done by the Department of Plant Sciences, Kunming Institute of Botany, Chinese Academy of Sciences.

4.2. Extraction procedure

The herb of *R. roseum* (1100 g) was successively infiltrated with 50% and 95% EtOH and H_2O respectively, at room temperature for 2 weeks and was evaporated under vacuum to obtain the different extracts (RE-50, RE-95 and RE-H_2O). The weight/weight yields in terms of crude medicinal materials was 0.13, 0.11 and 0.51 respectively. The extraction isolation of the plant materials, and the purification of the test substances uridine, caffeic acid methyl ester, methyl piperate, and piperine were carried out as described previously by Wang et al. (2005).

4.3. Chemicals

Isoproterenol, H_2O_2 and other chemicals were all of analytical grade and were obtained from Sigma Chemicals (St. Louis, MO). Double distilled water was used for all biochemical assays. The LDH, CK, MDA and SOD kits were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).

4.4. Experimental protocols

The experiments were carried out on Sprague-Dawley rats (150–200 g) treated humanely according to the 'Principles of Laboratory Animal Care' (NIH Publication No. 85-23, revised in 1985). 8–10 animals were kept in each cage under standard laboratory conditions (at a temperature of 20 ± 1 °C and a 12 h light/dark cycle) with free access to food and water. All experiments were performed from 9 : 00 am to 4 : 00 pm.

The animals were divided into 8 groups: the control group served as normal control and received distilled water (1 ml/kg, p.o.) daily for 11 days and in addition received distilled water (0.5 ml/kg, s.c.) on the 9th and 11th day at an interval of 24 h. The isoproterenol group served as ISO control and received distilled water (1 ml/kg, p.o.) daily for 11 days and in addition received ISO (85 mg/kg, s.c.) from 9th to the 11th day at an interval of 24 h. Experimental groups – termed as RE-50, RE-95 and RE-H_2O, received two dosages (250 mg/kg and 63 mg/kg, p.o.) daily for 11 days and in addition received ISO (85 mg/kg, s.c.) on the 9th and the 11th day at an interval of 24 h. The serum samples were obtained at the end of the experimental period, centrifuged and assayed for CK, GOT and LDH activities. The hearts were dissected immediately, washed with ice-cold saline and 10% homogenates in phosphate buffer (50 mM, pH 7.4) were prepared. The homogenates were centrifuged at $7000 \times g$ for 10 min at 4° and the supernatants were used for the MDA and SOD assays.

4.5. Measurement of serum enzyme levels in rats

The levels of marker enzymes CK, GOT and LDH in the serum, which are the diagnostic indicators of myocardial injury, were determined according to the instructions supplied with the assay kits. The SOD activity and MDA content in heart homogenates were measured according to the kit instructions using the method of Ohkawa et al. (1979) and Sun and Zigman (1978).

4.6. Neonatal cardiomyocyte isolation and H_2O_2-induced injury in cell

One- to 2-day-old neonatal Sprague-Dawley rats were used and each isolation was a single experimental replicate. Neonatal rats were briefly cleaned with 70% ethanol and the hearts were removed and minced in an enzyme solution containing collagenase type II (Gibico) and pancreatin (Sigma). Minced tissue and solutions were placed in a trypsinizing flask and shaken at 37 °C for 5 min to allow digestion after which the supernatant was collected and discarded. Ten milliliters of enzyme solution was added again to the flask and shaken at 37 °C for 15 min. The supernatant this time was retained in 50 ml tubes and centrifuged at 1200 rpm for 5 min. At the end, the cell pellet was resuspended in DMEM supplemented with 1% bovine serum albumin, 50 U/ml penicillin, and 50 mg/ml streptomycin 24 h prior to further treatments. Then, cardiomyocytes were treated with hydrogen peroxide (H_2O_2) (0.5 mM) for 4 h, and pretreated with different concentrations (10^{-4} M, 10^{-5} M, and 10^{-6} M) of test compounds. The medium was removed immediately before H_2O_2 exposure and cells were washed with PBS. This treatment protocol has been shown to induce ROS dependent gene expression without altering cell viability (Kemp et al. 2003). After 2 h, the medium was used to test CK and LDH.

4.7. Measurement of intracellular ROS

Intracellular oxidant stress was monitored by DCFH/DA (10 μM) using a modified method as previously described (Vanden Hoek et al. 1997a). Upon entry, DCFH/DA is cleaved by cellular esterases to nonfluorescent 2′,7′-dichlorofluorescin (DCFH) and oxidized by ROS to a fluorescent product dichlorofluorescein (DCF) which is measured at excitation 488 nm/emission 520 nm and expressed in arbitrary units (a.u.). Thus, the DCF fluorescence is directly proportional to the H_2O_2 and/or hydroxyl radical generation. Cardiac cells were loaded with DCFH/DA (10 μM) and exposed to H_2O_2 (0.5 mM) for 2 h or pretreated with methyl piperate, and piperine for 2 h prior to H_2O_2 exposure. An Olympus inverted phase/epi-fluorescent microscope was used for cell imaging. Fluorescent image changes in fluorescent intensity over time were quantified using Victor$^3_{TM}$ and its software (PerkinElmer, USA).

4.8. Data analysis

All data are expressed as means ± standard deviation (S.D.), and the level of significance between two groups was assessed with Student's t-test. P values of less than 0.01 were considered to be statistically significant.

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References

Bandyopadhyay D, Chattopadhyay A, Ghosh G, Datta AG (2004) Oxidative stress-induced ischemic heart disease: protection by antioxidants. Curr Med Chem 11: 369–387.

Carter WO, Narayanan PK, Robinson JP (1994) Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. J Leukoc Biol 55: 253–258.

Dilsiz N, Sahaboglu A, Yildiz MZ, Reichenbach A (2006) Protective effects of various antioxidants during ischemia-reperfusion in the rat retina. Graefe Arch Clin Exp Ophthalmol 244: 627–633.

Ding H (1982) Medicinal spore-bearing plants of China. Shanghai Press. p. 409

Eric SJ Harris (2006) The 47th Annual Meeting of Society for Economic Botany. The Lives of a Chinese Medicinal Moss: Variation and Standardization. Abstract 28. June 5–June 9, 2006

Fan LL, O'Keefe DD, Powell WJ (1984) Effect of puerarin on regional myocardial blood flow and cardiac hemodynamics in dogs with acute myocardial ischemia. Yao Xue Xue Bao 19: 801–807

Fridovich I (1986) Biological effects of the superoxide radical. Arch Biochem Biophys 247: 1–11.

Gürgün C, Ildızlı M, Yavuzgil O, Sin A, Apaydın A, Cınar C, Kültürsay H (2008) The effects of short term statin treatment on left ventricular function and inflammatory markers in patients with chronic heart failure. Int J Cardiol 123: 102–107.

Karthikeyan K, Bai BR, Devaraj SN (2007). Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. Int J Cardiol 115: 326–333.

Kemp TJ, Causton HC (2003) Clerk A Changes in gene expression induced by H_2O_2 in cardiac myocytes. Biochem Biophys Res Commun 307: 416–421.

Khan M, Varadharaj S, Ganesan LP, Shobha JC, Naidu MU, Parinandi NL, Tridandapani S, Kutala VK, Kuppusamy P (2006) C-phycocyanin protects against ischemia–reperfusion injury of heart through involvement of p 38 MAPK and ERK signaling. Am J Physiol.: Heart Circ Physiol 290: H 2136–H2145.

Lil JL, Stantman FW, Lardy HA (1988) Antioxidant enzyme systems in rat liver and skeletal muscle. Arch Biochem Biophys 263: 150–160.

Ohkawa H, Ohishi N, Yagi K (1979) Assay of lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358.

Pandry NR, Kaur G, Chandra M, Sanwal GG, Misra MK (2000) Enzymatic oxidant and antioxidants of human blood platelets in unstable angina and myocardial infarction. Int J Cardiol 76: 33–38.

Rona G, Chappel CI, Balazs T, Gaudry R (1959) An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch Pathol 76: 443–445.

Sabeena Farvin KH, Anandan R, Kumar SH, Shiny KS, Sankar TV, Thankappan TK (2004) Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. Pharmacol Res 50: 231–236.

Sawyer DB, Siwik DA, Xiao L, Pimentel DR, Singh K, Colucci WS (2002) Role of oxidative stress in myocardial hypertrophy and failure. J Mol Cell Cardiol 34: 379–388.

Sharma M, Kishore K, Gupta SK, Joshi S, Arya DS (2001) Cardioprotective potential of ocimum sanctum in isoproterenol induced myocardial infarction in rats. Mol Cell Biochem 225: 75–83.

Sun M, Zigman S (1978) An improved spectrophotometric assay for superoxide dismutase based on epinephrine auto-oxidation. Anal Biochem 90: 81–89.

Vanden Hoek TL, Li C, Shao Z, Schumacker PT, Becker LB (1997a) Significant levels of oxidants are generated by isolated cardiomyocytes during ischemia prior to reperfusion. J Mol Cell Cardiol 29: 2571–2583.

Wang Bo, Liu Ping, Shen Yue-mao, Dai Chang (2005) Studies on chemical constituents from herb of Rhodobryum roseum. Zhong Guo Zhong Yao Za Zhi 30(12): 895–897

Wolfe JT, Ross D, Cohen GM (1994) A role for metals and free radicals in the induction of apoptosis in thymocytes. FEBS Lett. 352: 58–62.

Wu PC (1982) Some uses of mosses in China. Bryol Times 13: 5.

Yogeeta SK, Gnanapragasam A, Kumar SS, Subhashini R, Sathivel A, Devaki T (2006) Synergistic interactions of ferulic acid with ascorbic acid: Its cardioprotetive role during isoproterenol-induced myocardial infarction in rats. Mol Cell Biochem 283: 139–46.

Young YS, Woodside JV (2001) Antioxidants in health and disease. J Clin Pathol 54: 176–86.

Yu Syueming, Ma Yuan (1993) Effect of *Rhodobryum roseum* on hemorheology following acute coronary occlusion in dogs. Zhongguo Zhong Xi Yi Jie He Za Zhi 13: 672–674, 646.

Yu Syueming, Ma Yuan, Xia Tian, Ma Shiping (1995) Studies of action of *Rhodobrun roseum limpr* on changes of red cell aggregation and yield-shear stress in dogs with acute experimental coronary occlusion. Zhongguo Zhongyao Zazhi 20: 429–431.

Zhang X, Azhar G, Nagano K, Wei JY (2001) Differential vulnerability to oxidative stress in rat cardiac myocytes versus fibroblasts. J Am Coll Cardiol 38: 2055–2062.

Zhou B, Wu LJ, Li LH, Tashiro S, Onodera S, Uchiumi F, Ikejima T (2006) Silibinin protects against isoproterenol-induced rat cardiac myocyte injury through mitochondrial pathway after up-regulation of SIRT1. J Pharmacol Sci 102: 387–395.