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MORIN, A FLAVONOID, ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN EXPERIMENTAL MYOCARDIAL ISCHEMIC RATS

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Abstract

Background: Myocardial infarction affects a large population in the world. Lipid peroxide metabolism plays an important role in the pathology of myocardial infarction. **Objective:** The present study was designed to investigate the antioxidant potential of morin, a flavonoid in isoproterenol (ISO)-induced myocardial infarction (MI), in rats. **Materials and Methods:** Male albino Wistar rats were pre-treated with morin (40 mg/kg), daily for a period of 30 days. After the treatment period, ISO (85 mg/kg), was subcutaneously injected in rats at an interval of 24 h for 2 days. **Results:** ISO-administered rats showed elevated levels of thiobarbituric acid reactive substances (TBARS), and lipid hydro-peroxide (LOOH), in plasma and heart. Pretreatment with morin, the above changes were significantly reduced to near normal level. ISO-administered rats showed decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in heart. In addition, decrease the levels non enzymatic antioxidants such as reduced glutathione (GSH), vitamin C and vitamin E in plasma and heart while ceruloplasmin in plasma. **Conclusion:** Pretreatment with morin, reversed these above biochemical changes towards normalcy. These findings revealed that, the morin possess antioxidant activity in experimentally induced cardiac toxicity.

Key words: Morin, Isoproterenol, Myocardial infarction, Lipid peroxidation, Antioxidants

Introduction

Myocardial infarction is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply which results in the necrosis of the myocardium (Anversa and Sonnenblick, 1990). This is usually followed by numerous pathophysiological and biochemical changes including lipid peroxidation and hyperlipidemia (Suchalatha and Shyamala-Devi, 1990). It has also been suggested that heart failure subsequent to myocardial infarction may be associated with antioxidant deficit as well as increased myocardial oxidative stress (Hill and Singal, 1996). Free radicals and reactive oxygen species have been implicated in large number of diseases and have a deleterious effect on heart functioning. Various experimental and clinical studies have shown that enormous amount of reactive oxygen species such as, superoxide, hydrogen peroxide and hydrogen radicals are generated in failing myocardium (Rajadurai and Prince, 2006). Therefore, therapeutic interventions having antioxidants or free radical scavenging activity may be useful against oxidative stress associated with various cardiovascular diseases including myocardial infarction.

Isoproterenol [1-(3, 4-dihydroxyphenyl)-2-isopropylaminoethanolhydrochloride], (Chagoya De Sanchez et al., 1997), is a synthetic catecholamine and beta-adrenergic agonist. The excess amount of ISO produces free radicals through its metabolites which are responsible for oxidative stress, and cardiac damage. The rat model of ISO-induced MI serves as a standard model to estimate the effect of cardio protective drugs in preclinical study and show many metabolic and morphologic alterations in the heart tissue of the experimental animals similar to those observed in human MI (Ithayarasi and Dev, 1997; Kukreja and Hess, 1992).

Many plants and plant derived compounds have been used in the treatment of MI in Ayurvedic Medicine. Plants constitute an important source of active natural products which differ widely in term of structure and biological properties. In recent years, the prevention of cardiovascular diseases (CVD) has been associated with the ingestion of fresh fruits vegetables or plant rich in natural antioxidants. Flavonoids are ubiquitous compounds which occur in plant sources like tea, herbs, citrus fruits and red wine. Several epidemiological studies have reported that the flavonoids reduced the risk of CVD (Chen et al., 1989; Sesso et al., 2003).

Morin (3, 5, 7, 2', 4'-pentahydroxyflavone; a yellowish pigment) is a bioflavonoid constituent of many herbs and fruits (Fig.1). Bioflavonoids are used as herbal medicines, and exhibit various biological activities including antioxidant cytoprotection, antimutagenesis and anti-inflammation (Francis et al., 1989). It was reported that morin could modulate the activities of the metabolic enzymes, including cytochrome P450 (Hodek et al., 2002), and it is also an antioxidant that protects various human cells, like myocytes, endothelial cells, hepatocytes and erythrocytes, against oxidative damages (Wu et al., 1993; Kitagawa et al., 2004). Moreover, morin acts as a chemopreventive agent against oral carcinogenesis *in vitro* and *in vivo* (Kawabata et al., 1999; Brown et al., 2003). Our study shows that pretreatment with morin, a flavonoid ameliorates adenosine triphosphatases and glycoproteins and exhibits beneficial role on cardiac mitochondrial function during ISO induced myocardial infarction in male Wistar rats (Al-Numair et al., 2012; Al-Numair et al., 2012). In view of the above facts, the present investigation was undertaken to study the antioxidant potential of morin in ISO induced myocardial infarction in male albino Wistar rats.

Materials and methods

Experimental Animals

Male albino rats of Wistar strain of body weight ranging from 140 to 160 g were procured from Central Animal House, King Saud University, and they were maintained in an air conditioned room (25 ± 1°C), with a 12h light/12h dark cycle. The animals were fed ad libitum with

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normal laboratory pellet diet and Procedures involving animals and their care were accordance with the Policy of Research Centre, King Saud University.

Drugs and Chemicals

Isoproterenol hydrochloride and morin were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Induction of Experimental Myocardial Infarction

Myocardial ischemia was induced by subcutaneous injection (s.c.) of isoproterenol hydrochloride (85 mg/kg BW, twice at an interval of 24h) for two consecutive days.

Experimental Design

In our earlier study conducted with three different doses of morin, (20, 40 and 80 mg/kg) to determine the dose dependent effect in ISO-treated rats. It was observed that morin pretreatment at doses of 40 mg/kg significantly ($P<0.05$), lowered elevated levels of creatine kinase (CK), creatine Kinase MB (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), in serum of ISO-induced rats after 30 days of experimental study than that of the other two doses (Al-Numair et al., 2012). Hence, at doses of 40 mg were chosen for this study.

The animals were randomly divided into four groups of six animals' each.—Group 1: control rats; Groups 2: normal rats treated with morin (40 mg/kg BW); Group 3: ISO control rats (85 mg/kg BW); Groups 4 rats pretreated with morin 40 mg/kg and then subcutaneously injected with ISO. Morin was dissolved in water and administered to rats orally using an intra-gastric tube daily for a period of 30 days and ISO (85 mg/kg), was subsequently injected in rats at an interval of 24 h for 2 days. After the last treatment, all the rats were sacrificed by cervical decapitation after an overnight fast. Blood was collected and serum and plasma separated by centrifugation. Heart tissue was excised immediately and rinsed in ice-chilled normal saline. A known weight of the heart tissue was homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH 7.4), solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical assays

The concentration of TBARS and LOOH were estimated by the method of Niehaus and Samuelson (1968), Fraga et al. (1988) and Jiang et al. (1992) respectively. The activity of SOD, CAT, GPx and GST were assayed by the method of Kakkar et al. (1984), Sinha (1972), Rotruck et al. (1973) and Habig and Jakoby (1981) respectively. GSH, Ascorbic acid and ά–tocopherol were determined by the method of Ellman (1959), Omaye et al. (1979), and Baker et al. (1980) respectively. Ceruloplasmin was determined using its copper oxidase activity by method of Ravin (1961). Protein in the tissues was determined after trichloroacetic acid precipitation by the method of Lowry et al. (1951).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT), using SPSS software package 9.05. Results were expressed as mean ± S.D. from six rats in each group. P values <0.05 were considered as significant.

Results

Effect of morin on TBARS and LOOH

Table 1 shows the levels of TBARS and LOOH in plasma and the heart of normal and experimental rats. Rats induced with ISO, showed a significant ($P<0.05$), increase in the levels of TBARS and LOOH in plasma and the heart when compared to normal control rats. Oral pretreatment with morin (40 mg/kg), to ISO-induced rats daily for a period of 30 days significantly ($P<0.05$), decreased the levels of TBARS and LOOH in plasma and the heart when compared with ISO-alone induced rats.

Effect of morin on SOD and CAT

Table 2 shows the activities of SOD and CAT in the heart of normal and experimental rats. Rats induced with ISO, exhibited a significant ($P < 0.05$), decrease in the activities of these antioxidant enzymes in the heart on comparison with normal control rats. Pretreatment with morin (40 mg/kg) to ISO-induced rats significantly ($P<0.05$) increased the activities of these enzymes when compared with ISO-alone induced rats.

Effect of morin on GPx, GST and GSH

Table 3 illustrates the effect of morin on the activities of myocardial GPx and GST and the levels of GSH in plasma and the heart in normal and ISO-induced rats. Rats induced with ISO, showed a significant ($P<0.05$), decrease in the activities of GPx and GST and the levels of GSH on comparison with normal control rats. Oral administration of morin (40 mg/kg) to ISO-induced rats significantly ($P<0.05$), increased the activities of these antioxidant enzymes and the levels of GSH when compared with ISO-alone induced rats.

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Table 1: Effect of morin on the levels of TBARS and LOOH in the plasma and heart of control and ischemic rats

Groups	TBARS		LOOH	
	Plasma (mmol/dL)	Heart (mmol/100g wet tissue)	Plasma ($X10^{-5}$ mmol/dL)	Heart (mmol/100g wet tissue)
Control	$0.17 \pm 0.01^{a,d}$	0.54 ± 0.03^{a}	$9.97 \pm 0.5^{a,d}$	$62.3 \pm 5.2^{a,d}$
Control + Morin (40 mg/kg BW)	0.13 ± 0.02^{b}	0.44 ± 0.02^{b}	9.03 ± 0.7^{b}	50.7 ± 4.3^{b}
Isoproterenol (85 mg/kg BW)	0.35 ± 0.02^{c}	1.56 ± 0.02^{c}	17.0 ± 1.2^{c}	108.6 ± 8.5^{c}
Morin (40 mg/kg BW) + Isoproterenol	0.19 ± 0.01^{d}	0.62 ± 0.06^{d}	10.54 ± 0.7^{d}	64.4 ± 3.7^{d}

Values are expressed as means ± S.D. for six rats in each group.
[a-d] Values not sharing a common superscript in a column differ significantly at $p<0.05$ (DMRT).
Percentage changes: Control + Morin and Isoproterenol alone group were compared with control.
ISO + Morin treated groups were compared with ISO alone.

Table 2: Effect morin on SOD and CAT activity in the heart of control and ischemic rats

Groups	SOD U^{*}/mg protein	CAT U^{∞}/mg protein
Control	6.11 ± 0.34^{a}	48.16 ± 2.75^{a}
Control + Morin (40 mg/kg BW)	6.31 ± 0.34^{a}	50.20 ± 2.41^{a}
Isoproterenol (85 mg/kg BW)	2.81 ± 0.22^{b}	25.39 ± 1.51^{b}
Morin (40 mg/kg BW) + Isoproterenol	5.75 ± 0.35^{c}	36.13 ± 2.27^{c}

Values are expressed as means ± S.D. for six rats in each group.; [a-c] Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT)
U^{*}- Enzyme concentration required for 50% inhibition of NBT reduction/minute; U^{∞}- µmole of hydrogen peroxide consumed/minute
Percentage changes: Control + Morin and Isoproterenol alone group were compared with control.; ISO + Morin treated groups were compared with ISO alone.

Effect of morin on vitamin C and E and ceruloplasmin

Table 4 shows the effect of morin on the levels of plasma and heart vitamin C and E and plasma ceruloplasmin in normal and ISO-induced rats. Rats induced with ISO, exhibited a significant (P<0.05), decrease in the levels of vitamin C and E in plasma and heart and ceruloplasmin in plasma when compared with normal control rats. Oral administration of morin (40 mg/kg), to ISO-induced rats significantly (P<0.05), increased the levels of vitamin C and E in plasma and the heart and ceruloplasmin in plasma when compared with ISO-alone induced rats.

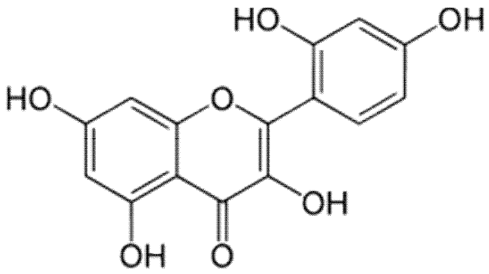

Figure 1: Structure of Morin

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Table 3: Effect of morin on the levels of GPx, GST and GSH in the plasma and heart of control and ischemic rats

Groups	Heart GPx U*/mg protein	Heart GST U#/mg protein	GSH Plasma (mg/dL)	GSH Heart (µg/mg protein)
Control	6.01± 0.33[a]	4.46 ± 0.18[a]	37.41 ± 2.42[a]	9.50 ± 0.59[a]
Control + Morin (40 mg/kg BW)	6.30 ± 0.32[b]	5.02 ± 0.24[b]	42.67 ± 3.62[b]	10.23 ± 0.63[a]
Isoproterenol (85 mg/kg BW)	2.65 ± 0.16[c]	2.01± 0.11[c]	17.20 ± 1.16[c]	4.87 ± 0.38[b]
Morin (40 mg/kg BW) + Isoproterenol	5.43 ± 0.29[d]	3.60 ± 0.11[d]	32.64 ± 1.23[d]	8.85 ± 0.38[c]

[a-d] Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT).; U*- µg of reduced glutathione consumed/minute
U #- µg of CDNB conjugate formed/minute; U*- µg of GSH formed/minute.; Percentage changes: Control + Morin and Isoproterenol alone group were compared with control.; ISO + Morin treated groups were compared with ISO alone.

Table 4: Effect of morin on the levels of vitamin c, vitamin e and ceruloplasminin the plasma and heart of control and ischemic rats

Groups	Vitamin C		Vitamin E		Ceruloplasmin
	Plasma (mg/dL)	Heart (µg/mg protein)	Plasma (mg/dL)	Heart (µg/mg protein)	Plasma (mg/dL)
Control	2.07 ± 0.22[a]	0.49 ± 0.015[a]	1.79 ± 0.16[a]	2.51 ± 0.16[a]	29.72 ± 1.98[a]
Control + Morin (40 mg/kg BW)	2.10 ± 0.24[a]	0.43 ± 0.030[a]	1.80 ± 0.20[a]	2.60 ± 0.26[a]	29.77 ± 1.88[a]
Isoproterenol (85 mg/kg BW)	1.02 ± 0.09[b]	0.14 ± 0.009[b]	0.72 ± 0.07[b]	1.10 ± 0.14[b]	18.57 ± 1.28[b]
Morin (40 mg/kg BW) + Isoproterenol	1.80 ± 0.13[c]	0.33 ± 0.019[c]	1.37 ± 0.08[c]	2.20 ± 0.12[c]	26.78 ± 2.17[c]

Values are expressed as means ± S.D. for six rats in each group.: [a-c] Values not sharing a common superscript in a column differ significantly at $p<0.05$ (DMRT).; Percentage changes: Control + Morin and Isoproterenol alone group were compared with control.
ISO + Morin treated groups were compared with ISO alone.

Discussion

Isoproterenol, a synthetic ß-adrenergic agonist by its positive inotropic and chronotropic actions, increases the myocardial oxygen demand that leads to ischemic necrosis of myocardium in rats. A number of pathophysiological mechanisms have been proposed to explain the ISO-induced myocardial damage, including altered permeability, increased turnover of norepinephrine, and generation of cytotoxic free radicals on autooxidation of catecholamine. Free radical-mediated lipid peroxidation and consequent changes in membrane permeability are the primary factors for cardio-toxicity induced by ISO (Noronha-Dutra et al., 1985). Oxidative stress increases cyclic adenosine monophosphate (cAMP), levels by exhausting adenosine

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triphosphate (ATP), and decreases sarcolemmal Ca^{+2} transports, resulting in intracellular calcium overload, which leads to ventricular dysfunction and contractile failure in rat heart (Bhagat et al., 1976; Tappia et al., 2001).

Lipid peroxidation is a well established mechanism of cellular injury and has been used as an indicator of oxidative stress. Increased levels of plasma and cardiac tissue thiobarbituric acid reactive substances and lipid hydro-peroxides levels indicate excessive production of free radicals and decreased antioxidant systems in myocardial infracted rats. In recent years, there is increasing interest in free radicals that have shown abilities to modify biological molecules, which may result in various pathological conditions (Maxwell, 1995). Thus, additional natural products need evaluation for their antioxidant potential. Lipid peroxidation, a type of oxidative deterioration of polyunsaturated fatty acid (PUFAs), has been linked with altered membrane structure and enzyme inactivation. ISO treatment showed an increase in the levels of TBARS and LOOH, in the plasma and heart. Increased lipid peroxidation appears to be the initial stage to the tissue making it more susceptible to oxidative damage. This may be responsible for the observed membrane damage as evidenced by the elevated lipid peroxidation in terms of TBARS and LOOH. Pretreatment with morin to ISO-induced rats significantly decreased the levels of TBARS and LOOH in both plasma and the heart. In this context, flavonoids have been to inhibit lipid peroxidation formation in rat tissues and also inhibit the free radical production in the cells at various stages. Previous studies reported that the flavonoids decrease the levels of lipid peroxidation products in isoproterenol-induced myocardial infarcted rats (Jayachandran et al., 2010; Murugesan and Manju, 2010). Thus, flavonoid scavenges the excessive free radicals produced by isoproterenol in myocardial infarcted rats and protects the myocardium, by its anti-lipid peroxidation effect. Wu et al. (1993), have reported that morin, a flavonoid to act as a potent antioxidant activity.

Reactive oxygen species (ROS) may injure cells by causing peroxidation of membrane lipid, denaturation of proteins including enzymes and ion channels, and strand breaks in deoxyribonucleic acid (DNA). Lipid peroxidation triggers loss of membrane integrity, necrosis and cell death (Park and Lucchesi, 1999). Oxidative damage by ROS has been documented in a number of experimental studies from subcellular and cellular to *in vitro* and *in vivo* models (Marczin et al., 2003), and also in humans (Ferrari et al., 1990; Ferrari et al., 1998). While low levels of oxygen radicals and oxidants are normally formed in cells and play important roles in cellular homeostasis, mitosis, differentiation, and signaling (Irani et al., 1997), following ischemia and reperfusion radical formation is greatly increased triggering cellular injury. Although mammalian cells including cardio-myocytes express endogenous free radical scavenging enzymes (Dhalla et al., 2000), such as superoxide dismutase, catalase, and glutathione peroxidase, these anti-oxidative defenses are overwhelmed after ischemia and reperfusion. Lipid peroxidation occurs upon ischemic injury, and can be prevented when oxygen radicals are inactivated by specific scavengers (Manning and Hearse, 1984; Zweier and Villamena, 2003). SOD and CAT are the first line of cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical. The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. In ISO-induced rats, the activities of heart tissue antioxidant enzymes were decreased. During MI, SOD and catalase are structurally and functionally impaired by free radicals resulting in myocardial damage. The decrease in SOD and catalase may be due to the involvement of superoxide and hydrogen peroxide free radicals in myocardial cell damage mediated by ISO (Guarnieri et al., 1980). Prior treatment with morin improved the activities of SOD and catalase by scavenging superoxide and hydrogen peroxides produced by ISO. Morin is a moderately potent inhibitor of xanthine oxidase (XO). In two separate assays of XO activity it was shown that morin is distinctly more inhibitory of this enzyme than Trolox but less so than allopurinol. XO is a key enzyme, especially in the vascular endothelium in many organs. It can generate a cascade of oxyradicals when these organs undergo ischemia-reperfusion (Flaherty and Weisfeldt, 1988). Morin can moderately inhibit XO implies that morin hydrate may act as a partially "preventive" antioxidant that militates against oxyradical generation, in addition to its ability to "cure" oxidative damage by scavenging oxyradicals.

GSH is an abundant and ubiquitous antioxidant, a tripeptide and essential biofactor synthesized in all living cells. It functions mainly as an effective intracellular reductant (Rahman and Macnee, 1999). It protects the cells from free radical mediated damage caused by drugs and ionizing radiation. It forms an important substrate for GPx, GST and several other enzymes, which is involved in the free radical scavenging action. In the heart, GPx is a major enzymatic mechanism for the disposal of peroxides, a prolonged depression in the activity of this enzyme may lead to the intracellular peroxide accumulation. GST acts like peroxidase and removes the stable peroxides from the system, resulting in the reduction of peroxide-induced damage (Jagetia et al., 2004). Decreased GSH levels might be due to increased utilization in protecting 'SH' containing proteins from lipid peroxides. The unavailability of GSH may decrease the activities of GPx and GST in ISO-induced rats. Pretreatment with morin significantly increased the concentration of GSH in plasma and the heart and the activities of GPx and GST in the heart of ISO-induced rats. Previous report shows that the morin offers to protect against hyperammonemia by reduced oxidative stress and enhanced antioxidant activity in ammonium chloride-induced hyperammonemic rats (Subhash and Subramanian, 2009).

The second line of defense consists of the non-enzymatic scavengers, namely, ascorbic acid, a-tocopherol, ceruloplasmin, and sulfhydryl-containing compounds, which scavenge the residual free radicals escaping from decomposition by the antioxidant enzymes. Decreased concentration of vitamin C and E in plasma and the heart and ceruloplasmin in plasma in ISO-induced rats were observed in our study. Vitamin C is a primary antioxidant, water-soluble vitamin that can directly scavenge singlet oxygen, superoxide and hydroxyl radicals. It has been suggested to reduce the risk of CVD by reducing blood pressure, blood cholesterol and the formation of oxidized low-density lipoprotein–cholesterol (Benidich et al., 1986). Vitamin E appears to be the most effective lipid soluble antioxidant in the biological system. It inhibits lipid peroxidation and regenerates reduced vitamin C and GSH. By protecting myocardial membranes and inhibiting the oxidation of lipoproteins, vitamin E inhibits membrane peroxidative damage and atherogenesis (Upston et al., 1999). Ceruloplasmin inhibits ferritin-dependent lipid peroxidation by catalyzing the oxidative reincorporation of released irons into ferritin (Sreedharan et al., 2009). Pretreatment with morin to ISO-induced rats significantly increased the levels of vitamin C and E in both plasma and heart and ceruloplasmin levels in plasma. In this context, the chemopreventive efficacy of morin on tissue lipid peroxidation and antioxidant status, which are used as biomarkers in 1,2-dimethylhydrazine-induced colon carcinogenesis in a rat model (Samokyszyn et al., 1989). Flavonoid antioxidants function as scavengers of free radicals by rapid donation of hydrogen atom to radicals (Amy et al., 2003). Wu et al. (1994), that morin hydrate can effectively protect against oxyradical damage in rabbit heart during ischemia-reperfusion through multiple mechanisms. Morin may inhibit oxyradical generation by inhibiting XO and/or chelating one or more metal ions such as Fe^{2+} in the cell or organ. It may also denote an electron to the oxyradical generated in the organ, forming a stable morin conjugated system. Flavonoids retain their free radical scavenging activities after forming complexes with iron ions and thus formation of metal ion chelates is also one of the antioxidant mechanisms of flavonoids (Cook and Samman, 1996).

Conclusion

This study demonstrates that morin protected the myocardium against ISO-induced infarction and suggest that these cardioprotective effects could be due to prevention or inhibition of lipid peroxidative system by its antioxidant effect.

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