Journal of Medicinal Plants Research Vol. 6(18), pp. 3445-3449, 16 May, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1760
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Morin, a flavonoid, prevents lysosomal damage in experimental myocardial ischemic rats

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Accepted 7 March, 2012

The present study was designed to investigate the preventive effect of morin on lysosomal enzymes in isoproterenol (ISO) treated myocardial infarcted rats. Myocardial ischemia was induced by subcutaneous injection of ISO hydrochloride (85 mg/kg BW, twice at an interval of 24 h) for two consecutive days. The morin (40 mg/kg BW) was administered daily for 30 days and subsequently two doses of ISO administered on 29th and 30th days. The activities of lysosomal enzymes β-glucuronidase, β-N-acetylglucosaminidase, β-galactosidase, cathepsin-B and D were increased significantly (P<0.05) in the serum and heart of ISO-induced cardiotoxic rats. The pretreatment of morin and two doses ISO treated rats exhibited significant (P<0.05) reduction of these lysosomal enzymes activities. Morin protects the lysosomal membrane damage against ISO-induced cardiac damage as evidenced by reduced activities of these lysosomal enzymes.

Key words: Morin, isoproterenol, myocardial infarction, lipid peroxidation, lysosomal enzymes.

INTRODUCTION

Heart ischemia is one of the main causes related to sudden death in the world. In the past decades, there has been a great increase in the use of complementary treatments such as herbal remedies in the treatment of various diseases. Many traditional plants and their active principles have claimed to be useful for the control of ischemia and associated pathologies. Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. ISO causes severe stress in the myocardial tissue of animals (Chagoya de Sanchez et al., 1997). Milei et al. (1978) have reported that ISO induces myocardial infarction in animals due to the action on the sarcolemmal membrane, stimulation of adenylate cyclase, activation of Na+ and Ca2+ channels and exaggerated Ca2+ inflow and energy consumption leading to cellular death. There is a report showing that free radicals produced by ISO could initiate peroxidation of membrane bound polyunsaturated fatty acids, leading to both functional and structural myocardial injury (Thompson and Hess, 1986). ISO induced myocardial infarction is a well standardized model to study the protective effects of many drugs and cardiac function, since it mimics the clinical conditions of myocardial infarction due to ischemia in humans (Ithayarasi and Devi, 1997). Among the various mechanisms proposed to explain ISO induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factors.

ISO also increases the activities of lysosomal enzymes. ISO induced myocardial infarction has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction (Nirmala and Puvanakrishnan, 1996). Lysosomes are membrane bound structures that contain hydrolytic enzymes capable of degrading most of the cellular constituents. In addition, lysosomes play a major role in secretion and transport processes. It has been postulated that the intracellular release of lysosomal enzymes and their subsequent extra lysosomal activity may exercise a pivotal role in the progressive

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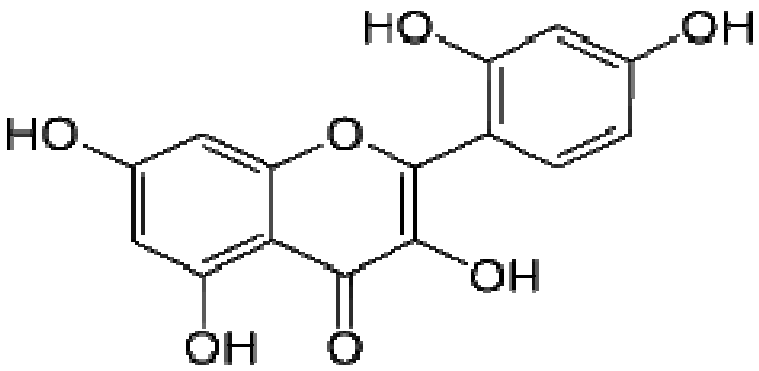

Figure 1. Chemical structure of morin.

modifications that lead from reversible myocardial ischaemia to irreversible infarction (Decker et al., 1977). ISO, a synthetic catecholamine and β-adrenergic agonist, causes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscle (Sushamakumari et al., 1989). ISO-induced myocardial infarction results in increased lysosomal hydrolases activity that may be responsible for tissue damage and infracted heart (Ravichandran et al., 1991). ISO-induced myocardial necrosis involves membrane permeability alterations that bring about loss of function and integrity of myocardial membranes (Todd et al., 1980).

Plants constitute an important source of active natural products which differ widely in terms of structure and biological properties. They have a remarkable role in the traditional medicine in different countries. In recent years, the prevention of cardiovascular diseases has been associated with ingestion of fresh fruits, vegetables or plants rich in natural antioxidants. The protective effects of plants can be due to the presence of flavonoids (Zhang and Wang, 2002), anthocyanins and other phenolic compounds (Sanchez-Moreno et al., 1998). Plant derived polyphenolic compounds possess a wide range of pharmacological properties and the study of their mechanism of action has been the subject of considerable interest in recent years. Morin (3, 5, 7, 2', 4'-pentahydroxyflavone; a yellowish pigment) is a bioflavonoid constituent of many herbs and fruits (Figure 1). Bioflavonoids are used as herbal medicines, and exhibit various biological activities including antioxidation, cytoprotection, antimutagenesis and anti-inflammation (Francis et al., 1989; Fang et al., 2003).

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 (Khalid et al., 2012) exhibits beneficial role on cardiac mitochondrial function during ISO induced myocardial infarction in male Wistar rats (Khalid et al., 2012). In view of the above facts, the present investigation was undertaken to study the preventive effect of morin in reducing the extent of lysosomal damage in the myocardium by virtue of its free radical scavenging and antioxidant properties in experimentally induced cardiotoxic 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℃) with a 12 h light/12 h dark cycle. The animal s were fed *ad libitum* with 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

Morin, ISO hydrochloride, N-phenyl-p-phenylenediamine, p-nitrophenyl-N-acetyl-β-D-glucosaminide, p-nitrophenyl-β-Dglucuronide, α-N-benzoyl-DL-arginine-p-nitroanilide hydrochloride and sodium dodecyl sulphate were purchased from Sigma Chemical Company. St. Louis, MO, USA. All other chemicals used were of analytical grade.

Induction of experimental myocardial infarction

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

Experimental design

In our earlier study we conducted three different doses of morin (20, 40 and 80 mg/kg) to determine the dose dependent effect in ISO-

Table 1. Effect of morin on the activities of β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase in the serum of control and ISO induced myocardial infarcted rats.

Group	β-glucuronidase (µmol of p-nitrophenol liberated/h/mg protein)	β-N-acetyl glucosaminidase (µmol of p-nitrophenol liberated/h/mg protein)	β-galactosidase (µmol of p-nitrophenol liberated/h/mg protein)
Control	9.16 ± 0.45[a]	16.24 ± 0.55[a]	7.79 ± 0.43[a]
Control + Morin (40 mg/kg/d)	8.99 ± 0.37[a]	16.00 ± 0.78[a]	7.74 ± 0.36[a]
ISO (85 mg/kg/d)	13.53 ± 0.69[b]	25.37 ± 1.34[b]	11.33 ± 0.36[b]
Morin (40 mg/kg/d) + ISO	10.47 ± 0.38[c]	18.55 ± 0.62[c]	8.45 ± 0.32[c]

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript in a column differ significantly at $p<0.05$ (DMRT).

Table 2. Effect of morin on the activities of β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase in the heart of control and ISO induced myocardial infarcted rats.

Group	β-glucuronidase (µmol of p-nitrophenol liberated/h/mg protein)	β-N-acetyl glucosaminidase (µmol of p-nitrophenol liberated/h/mg protein)	β-galactosidase (µmol of p-nitrophenol liberated/h/mg protein)
Control	10.51 ± 0.53[a]	31.20 ± 1.66[a]	12.01 ± 0.72[a]
Control + Morin (40 mg/kg/d)	10.33 ± 0.44[a]	30.74 ± 1.37[a]	11.63 ± 0.58[a]
ISO (85 mg/kg/d)	16.35 ± 0.57[b]	51.22 ± 2.53[b]	19.49 ± 0.48[b]
Morin (40 mg/kg/d) + ISO	12.55 ± 0.43[c]	36.09 ± 1.62[c]	13.43 ± 0.43[c]

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript in a column differ significantly at $p<0.05$ (DMRT).

treated rats. It was observed that morin pretreatment at dose of 40 mg/kg significantly (P<0.05) lowered elevated levels of creatine kinase (CK), creatine kinase MB (CK-MB), lactic dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum of ISO-induced rats after 30 days of experimental study than that of the other two doses (Khalid et al., 2012). Hence, dose of 40 mg was 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 alone cardiotoxic control rats (85 mg/kg BW); Groups 4: Pretreated with morin (40 mg/kg) and ISO administered rats. Morin was dissolved in water and administered to rats orally using an intragastric tube daily for 30 days and subsequently ISO treated with IP (85 mg/kg, s.c.) on 29th and 30th day in normal saline (Nandave et al., 2009). After the last treatment, all the rats were sacrificed by cervical decapitation after an overnight fast. Blood was collected and serum 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.

Assay of lysosomal enzymes

The activities of β-glucuronidase, β-N-acetyl glucos-aminidase and β-galactosidase were assayed (Kawai and Anno, 1971; Moore and Morris, 1982; Conchie et al., 1967), respectively. Activities of Cathepsin-B and D were assayed (Barrett, 1972; Sapolsky et al., 1973), respectively. The content of protein in the heart and subcellular fractions were determined (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

Table 1 shows the effect of morin on the activities of lysosomal enzymes β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase in serum of normal and ISO-induced rats. The activities of β-glucuronidase, β-N-acetyl glucos-aminidase and β-galactosidase were significantly (P<0.05) increased in the serum of ISO treated cardiotoxic rats compared to normal control rats. The pretreatment of morin (40 mg/kg) for 30 days and two doses of ISO (85 mg/kg BW) administered rats showed significant (P<0.05) reduction of these enzymes activities. Table 2

Table 3. Effect of morin on the activities of cathepsin-B and cathepsin-D in the serum and heart of control and ISO induced myocardial infarcted rats.

Group	Serum		Heart	
	Cathepsin-B (µmol of p-nitrophenol liberated/h/100 mg protein)	Cathepsin-D (µmol of tyrosine liberated/h/100 mg protein)	Cathepsin-B (µmol of p-nitrophenol liberated/h/100 mg protein)	Cathepsin-D (µmol of tyrosine liberated/h/100 mg protein)
Control	12.66 ± 0.38[a]	12.74 ± 0.63[a]	20.61 ± 0.95[a]	13.52 ± 0.54[a]
Control + Morin (40 mg/kg/d)	12.23 ± 0.52[a]	12.25 ± 0.47[a]	19.96 ± 1.11[a]	13.00 ± 0.60[a]
ISO (85 mg/kg/d)	18.62 ± 0.54[b]	17.99 ± 0.54[b]	31.55 ± 1.21[b]	19.78 ± 0.79[b]
Morin (40 mg/kg/d) + ISO	14.53 ± 0.32[c]	14.60 ± 0.31[c]	23.21 ± 1.02[c]	15.01 ± 0.43[c]

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript in a column differ significantly at $p<0.05$ (DMRT).

shows the effect of morin on the activities of lysosomal enzymes β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase in heart of normal and ISO-induced rats. In ISO-induced rats the activities of β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase were significantly ($P<0.05$) increased in the heart compared to normal control rats while pretreatment with morin (40 mg/kg) for 30 days and two doses of ISO (85 mg/kg BW) administered rats exhibited significant ($P<0.05$) reduction of these enzymes activities. Table 3 shows the effect of morin on the activities of cathepsin-B and D in serum and heart on normal and ISO-induced rats. The activities of cathepsin-B and D were significantly ($P<0.05$) increased in the serum and heart of ISO treated cardiotoxic rats compared to normal control rats. The pretreatment of morin (40 mg/kg) daily for 30 days and ISO (85 mg/kg BW) administration for two doses showed significantly reduce the activities of these enzymes when compared to ISO alone induced cardiotoxic rats.

DISCUSSION

The lysosomal compartment is the major site of intracellular protein degradation. Its interior is acidic and contains numerous hydrolytic enzymes that can degrade nearly all cellular components (Sudharsan et al., 2006). Administration of ISO to rats leads to a significant elevation of lysosomal enzymes activities in the serum and myocardium. Intracellular release of lysosomal enzymes following myocardial ischemia may directly (David and Marisol, 2000) or through activation of the complement pathway result in cell injury and death. It has been suggested that 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).

Lysosomal membrane plays a vital role in the regulation of lysosomal enzyme secretion in pathophysiology and in various inflammatory conditions (Pillay et al., 2002). Increased activities of lysosomal enzymes β-glucuronidase, β-N-acetyl glucosaminidase, β-galactosidase, cathepsin-B and D were observed in the serum and heart of ISO treated rats. Increased lipid peroxidation observed in ISO treated rats could have resulted in the leakage of serum and myocardial acid hydrolases from the enclosed sacs due to lysosomal membrane damage (Macickova et al., 1999).

It has been shown that the cytosolic acid hydrolases released from lysosomes and from the sarcoplasmic reticulum induce the dysfunction and destruction of mitochondria, sarcolemma and other organelles (Kennett and Weglicki, 1978). Pretreatment with morin normalized the activities of lysosomal enzymes both in the serum and myocardium by its inhibitory effect on lipid peroxidation, thereby preventing lysosomal damage induced by ISO treated rats. In this context, previous study (Decharneux et al., 1992) proposed a more direct action of flavonoids on lysosomal enzymes activities. Current evidence proved that morin, a flavonoid, modulates the activities of the metabolic enzymes, including cytochrome P450, and it is also an antioxidant that protects various human cells, like myocytes, endothelial cells, hepatocytes and erythrocytes, against oxidative damage (Wu et al., 1993; Kitagawa et al., 2004). Additionally, the localization of flavonoids within the membranes may modify membrane fluidity and lipid peroxidation as well documented.

Conclusion

Thus, the findings of this study show that morin

preserves the integrity of lysosomal membrane by maintaining the activities of lysosomal enzymes in serum and heart ISO-induced rats. This effect is due to the free radical scavenging and membrane stabilizing actions of morin.

ACKNOWLEDGEMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Applied Medical Sciences Research Center. Authors would like to express thank for support from the National Nutrition Policy Chair, King Saud University, Riyadh, Saudi Arabia.

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