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Original Article

Efficacy of morin on serum and heart tissue lipids in rats subjected to isoproterenol-induced myocardial injury

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Summary. Lipids and lipoproteins play an important role in the pathology of myocardial infarction. In our early study shows, pretreatment with morin, a flavonoid, ameliorates cardiac markers, adenosine triphosphatases and glycoprotein's in isoproterenol (ISO) induced myocardial infarction in rats at the optimum dose of 40 mg/kg BW. The present study evaluated the flavonoid-morin on lipid profiles in isoproterenol (ISO) induced myocardial infarction in rats. Male albino Wistar rats were pretreated with morin at the optimum dose of 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. ISO caused a significant increase in the activity of total cholesterol (TC), cholesterol ester (CE), free cholesterol (FC), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) in serum and decreased activities in heart phospholipids, serum high density lipoprotein-cholesterol (HDL-C) levels. Pre-treatment with morin at 40 mg/kg dose blocked these changes to normality which proved their antihyperlipidemic action. These findings provided evidence that morin was found to be protecting the myocardium against ischemic insult and the protective effect could attribute to its antihyperlipidemic activities.

Key words: Isoproterenol, myocardial infarction, lipids, antihyperlipidemic, morin, flavonoid

«L'effetto di morin sui lipidi sierici e del tessuto cardiaco in ratti sottoposti a danno miocardico isoproterenolo-indotto»

Riassunto. I lipidi e le lipoproteine svolgono un ruolo importante nella patologia dell'infarto miocardico. Nel nostro studio si evidenzia come il pretrattamento con morin, un flavonoide, migliora i marcatori cardiaci, l'adenosina trifosfatasi e le glicoproteine in ratti con infarto miocardico isoproterenolo (ISO) indotto alla dose ottimale di 40 mg/kg di peso corporeo. Il presente studio ha valutato il flavonoide-morin sui profili lipidici di ratti colpiti da infarto miocardico ISO-indotto. Ratti Wistar maschi albini sono stati pretrattati con morin alla dose ottimale di 40 mg/kg al giorno per un periodo di 30 giorni. Dopo il periodo di trattamento nei ratti è stato iniettato per via sottocutanea ISO (85 mg/kg) ad un intervallo di 24 h per 2 giorni. ISO ha provocato un notevole incremento nell'attività di colesterolo totale (TC), esteri del colesterolo (CE), colesterolo libero (FC), trigliceridi (TG), acidi grassi liberi (FFA) e fosfolipidi (PL), lipoproteine a bassa densità (C-LDL) e lipoproteine a densità molto bassa (C-VLDL) nel siero e ha diminuito l'attività nei fosfolipidi cardiaci e i livelli di lipoproteine ad alta densità (C-HDL) del siero. Il pre-trattamento con morin alla dose di 40 mg/kg ha bloccato questi cambiamenti verso la normalità dimostrandone l'azione antiperlipidemica. Questi risultati dimostrano che morin sembra proteggere il miocardio dai danni ischemici e l'effetto protettivo potrebbe essere attribuito alla sua attività antiiperlipidemica.

Parole chiave: Isoproterenolo, infarto miocardico, lipidi, antiperlipidemico, morin, flavonoide

Introduction

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. The World Health Organization (WHO) estimates that 17 million people die of cardiovascular disease annually (1). WHO predicts, that deaths due to circulatory system diseases are projected to double by 2015 (2). Myocardial infarction (MI) is the rapid development of myocardial necrosis caused by critical imbalance between the oxygen supply and the demand of the myocardium. A catecholamine, isoproterenol (ISO) is used to study the protective effect of various drugs on cardiac function. MI induced by ISO, a β-adrenergic agonist has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human MI (3). Accumulation of lipids in the myocardium might be due to the enhanced activity of adenylate cyclase in ISO treated rats (4). And ISO causes an increase in the levels of circulatory and myocardial lipids (5). An increased risk of coronary heart disease (CHD) is associated with high levels of serum TC and LDL-C and decreased levels of HDL-C. Increased LDL-C in circulation leads to accumulation of harmful deposits in the arteries thus favoring coronary heart diseases (6).

Flavonoids are a family of diphenylpropanes most commonly found in a variety of fruits, vegetables, juices, and components of herbal containing dietary supplements. Morin (Fig. 1) (3,5,7,2′,4′-pentahydroxyflavone) is a member of the flavonoid family which consists of a yellowish pigment found in mill (*Prunus dulcis*), (*Chlorophora tinctoria*), and other moraceae used as food and herbal medicine (7). Moreover, morin has been reported to possess a variety of biological properties against oxidative stress-induced damage, including the protection of cardiovascular cells (8), glomerular mesangial cells (9), hepatocytes (10), oligodendrocytes, and neurons (11, 12) damaged by oxidative stress. Circulating lipids and lipoproteins play an important role in the pathogenesis of myocardial infarction. In our early study shows, pretreatment with morin, a flavonoid, ameliorates cardiac markers, adenosine triphosphatases and glycoproteins in ISO-induced myocardial infarction in rats at the optimum dose of 40 mg/kg BW (13) and also exhibits beneficial role on cardiac mitochondrial function during ISO-induced myocardial infarction in male Wistar rats (14). To achieve the greatest possible reduction in myocardial infarction risk, treatment strategies should be aimed at reducing the elevated levels of circulatory lipids and correcting the levels of lipoproteins. The aim of the present study was to investigate the preventive effects of morin to ameliorate the lipid metabolism on ISO-induced-myocardial infarction in rats.

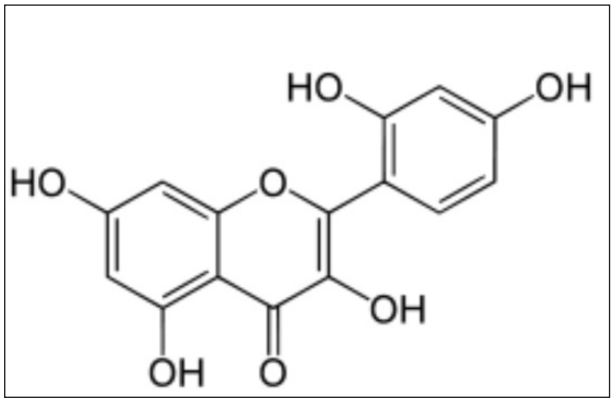


Figure 1. Structure of Morin

Material and methods

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 12 h light/12 h dark cycle. The animals were fed *ad libitum* with normal laboratory pellet diet and procedures involving animals and their care were in accordance with the Policy of Research Centre, King Saud University.

Chemicals

Isoproterenol hydrochloride and Morin hydrate (3,5,7,2′,4′-pentahydroxyflavone) of 95% purity was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Induction of myocardial ischemia

Myocardial ischemia was induced by subcutaneous injection (s.c.) of ISO (85 mg/kg BW, twice at an interval of 24 h) for two consecutive days (15).

Experimental design

The animals were randomly divided into four groups of ten animals each. Group 1: control rats (injected with saline); 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 at the optimum dose of 40 mg/kg BW) and then subcutaneously injected with ISO. Morin was dissolved in water and administered to rats orally using an intragastric tube daily for a period of 30 days and subsequently treated with ISO (85mg/kg, s.c.) on 29th and 30th day in normal saline (16).

Group I: control

Group II: control + Morin (40 mg/kg BW)

Group III: control + ISO (85 mg/kg BW)

Group VI: control + ISO (85 mg/kg BW) + Morin (40 mg/kg BW)

At the end of the experimental period, rats were anaesthetized with an intramuscular injection of ketamine hydrochloride (24 mg/kg BW), and sacrificed by cervical dislocation. Blood was collected, centrifuged at 2000 rpm for 10min and the separated serum was used for lipid estimations. After the tissues (heart) were excised, washed in ice-cold isotonic saline and blotted with a filter paper. A portion of the tissue was weighed, homogenized in 0.1M Tris–HCl buffer (pH 7.4) and the homogenate was used for tissue lipid estimations.

Biochemical estimation

Serum and tissue lipids were extracted as described previously (17). To a known volume of serum or tissue homogenate, 10.0 mL of chloroform–methanol (2:1, v/v) mixture was added and mixed well for 30 min and was filtered through Whatmann filter paper (No.42) into a separating funnel. The filtrate was mixed with 0.2 mL of physiological saline and the mixture was kept undisturbed overnight. The lower phase containing the lipid was drained off into pre-weighed beakers. The upper phase was re-extracted with more of chloroform–methanol mixture; the extracts were pooled and evaporated under vacuum at room temperature. The lipid extract was re-dissolved in 3.0 mL of chloroform–methanol (2:1) mixture and aliquots were taken for the estimation of lipids. Total cholesterol (TC) (18), free cholesterol (FC) and cholesterol ester (CE) (19), triglycerides (TG) (20), free fatty acids (FFA) (21), and phospholipids (PL) (22) were estimated as described previously. High density lipoprotein-cholesterol (HDL-C) (23) and low density lipoprotein-cholesterol (LDL-C) (24), very low density lipoprotein cholesterol (VLDL-C) (24) in serum were estimated by methods previously described. LDL-C and VLDL-C fractions were calculated as VLDL-C=TG/5 and LDL-C=total cholesterol– (HDL-C +VLDL-C), respectively.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package10.0. Results were expressed as means ± S.D. from 10 rats in each group. P values < 0.05 were considered as significant.

Results and discussion

Lipids play an important role in cardiovascular disease, and high levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage (25, 26). An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death that follows coronary artery occlusion (27). The cardiac muscle generally utilizes fatty acid as the major source of energy of the total oxygen consumption; 60–90% is utilized to oxidize fatty acid under aerobic condition. Under anoxic conditions, the cardiac muscle is not in a position to oxidize the available fatty acids, as a result of which there is an increase in the levels of these long chain fatty acyl CoA derivatives (28). In this study table 1 and 2 show effect of morin on the levels of TC, CE and FC, TG, PL,

Table 1. Effect of morin on the levels of total, ester and free cholesterol in serum and heart of control and ischemic rats.

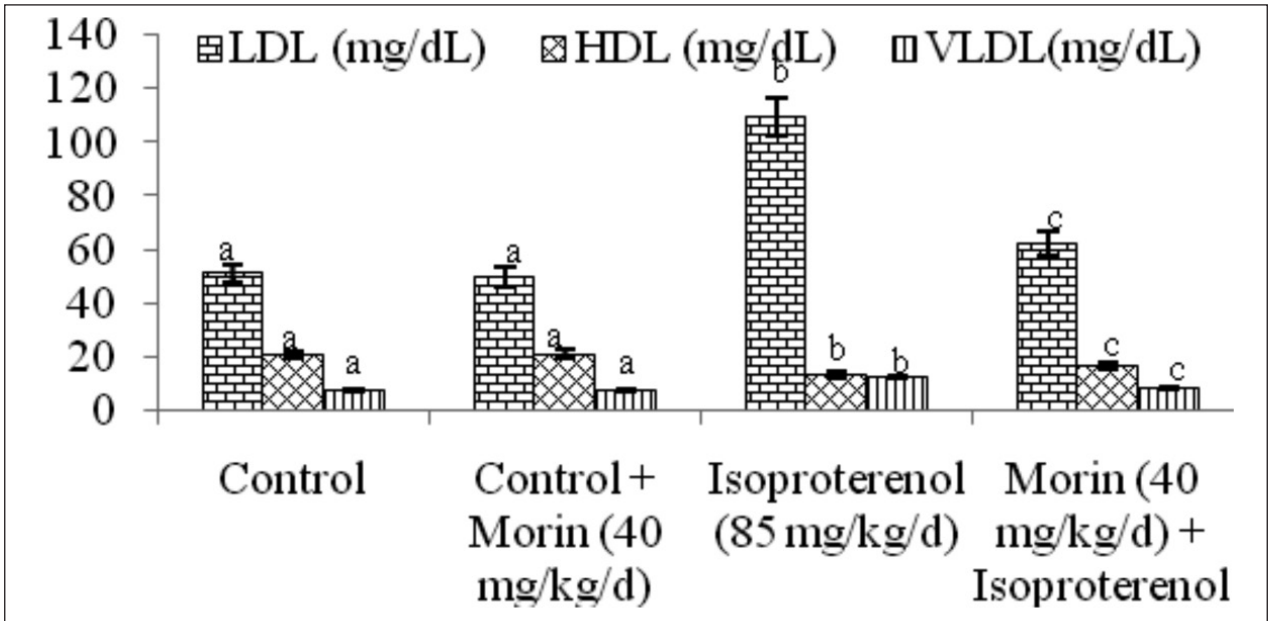
Groups	Total cholesterol		Ester cholesterol		Free cholesterol	
	Serum (mg/dL)	Heart (mg/g wet tissue)	Serum (mg/dL)	Heart (mg/g wet tissue)	Serum (mg/dL)	Heart (mg/g wet tissue)
Control	83.11 ± 5.11a	6.70 ±0.51a	51.05 ± 3.15a	4.15 ± 0.19a	27.34 ± 1.93a	2.72 ± 0.09a
Control + Morin (40 mg/kg/d)	80.65 ± 5.02a	6.58 ± 0.48a	49.85 ± 3.08a	4.02 ± 0.16 a	26.59 ± 1.54a	2.59 ± 0.10a
Isoproterenol (85 mg/kg/d)	135.11 ± 7. 63b	11.45 ± 0.65b	78.34 ± 4.66b	7.10 ± 0.68 b	52.15 ± 3. 46b	5.32 ± 0.25 b
Morin (40 mg/kg/d) + Isoproterenol	90.10 ± 7.12c	7.30 ± 0.51c	56.55 ± 3.85c	5.21 ± 0.37b	32.28 ± 2.08c	3.27 ± 0.14c

Values are expressed as means ± S.D. for ten rats in each group.
[a-c]Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

Table 2. Effect of morin on the levels of free fatty acids, phospholipids and triglycerides in the serum and heart of control and ischemic rats

Groups	Free fatty acids		Phospholipids		Triglycerides	
	Serum (mg/dL)	Heart (mg/g wet tissue)	Serum (mg/dL)	Heart (mg/g wet tissue)	Serum (mg/dL)	Heart (mg/g wet tissue)
Control	28.63 ±1.61a	0.27 ± 0.02a	78.52 ± 5.11a	29.16 ± 1.67a	40.34 ± 2.14a	3.54 ± 0.21a
Control + Morin (40 mg/kg/d)	27.94 ± 1.49a	0.25 ± 0.02a	77.01 ± 4.96a	30.02 ± 1.89a	39.65 ± 2.02a	3.41 ± 0.19a
Isoproterenol (85 mg/kg/d)	47.89 ± 2.96b	0.52 ± 0.03b	95.12 ± 6.35b	18.19 ± 1.10b	67.81 ± 3.24b	5. 67 ± 0.31 b
Morin (40 mg/kg/d) + Isoproterenol	32.16 ± 1.69c	0.32 ± 0.02c	74.21 ± 4.18c	26.48 ± 1.51c	48.09 ± 2.96c	4.16 ± 0.22c

Values are expressed as means ± S.D. for ten rats in each group.
[a-c]Values not sharing a common superscript differ significantly at p<0.05 (DMRT)



Values are expressed as means ± S.D. for 10 rats in each group.
[a-c]Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

Figure 2. Effect of morin on the levels of LDL, HDL and VLDL in the serum of control and ischemic rats.

and FFA in serum and heart of control and ischemic rats. In ISO-induced rats showed increased levels of TC, CE, FC, TG, PL and FFA in serum and heart of rats and on treatment with morin (40 mg/kg BW), the levels of these parameters brought towards normality. Accelerated degradation of membrane PL is very likely the biochemical basis for the irreversible cell injury in myocardial ischemia (29).

In the present study figure 2 shows the effect of morin on the levels of LDL, HDL and VLDL in the serum of control and ischemic rats. The increased levels of serum LDL and VLDL, while decreased level of HDL cholesterol were observed in ISO-induced rats. On treatment with morin (40 mg/kg BW), the levels of these parameters brought towards normality. These changes could be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate (30). High levels of LDL cholesterol show a positive correlation with myocardial infarction, whereas high levels of HDL cholesterol have a negative correlation (31). ISO-induced rats showed an increase in serum phospholipids, which may be due to an increased peroxidation of membrane phospholipids released via phospholipase A2 (32). Morin, exhibits significant beneficial effect on blood pressure, lipid profiles, and serum insulin and glucose in HF-induced hypertensive rats (33). In our present study pretreatment with morin showed a significant effect on all lipid parameters in the treatment group rats when compared to isoproterenol-administered rats. This effect might be due to the free radical scavenging and antioxidant property of morin (34). Morin is a moderately potent inhibitor of xanthine oxidase (35) it 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.

In the present study, pretreatment with morin showed to attenuate the alterations of myocardial lipids. Previous report shows, preventive effect of s-allylcysteine and α-tocopherol (positive control) on lipids in normal and isoproterenol-induced cardiac toxicity in male wistar rats (36). Previously Morin has been reported to protect 1,2-dimethylhydrazine induced oxidative stress due to its antioxidant efficacy (37). Morin contains five hydroxyl groups in the aromatic ring system. 2', 4' hydroxyl configuration in the B ring, require for scavenging free radicals. Thus the presence of hydroxyl groups at positions 2', 4' may be the responsible for the cardio protective effect of morin by donating/accepting electrons with the free radicals generated due to ISO induction.

Thus our findings demonstrate that morin exhibits cardioprotective effect in ISO-induced MI in rats. The pretreatment with morin has played a major role in lipid metabolism that may be due to its anti-lipidperoxidative effect and the present study strongly supports the efficacy of morin in controlling lipid parameters in serum and heart of ISO-induced myocardial infarction in rats.

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