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Pretreatment with morin, a flavonoid, ameliorates adenosine triphosphatases and glycoproteins in isoproterenol-induced myocardial infarction in rats

Khalid S. Al-Numair · Govindasamy Chandramohan · Mohammed A. Alsaif

Abstract The aim of this investigation was to evaluate the preventive role of morin, a flavonoid, on cardiac marker enzymes such as aspartate transaminase, lactate dehydrogenase, creatine kinase and creatine kinase-MB, membrane-bound enzymes such as sodium potassium-dependent adenosine triphosphatase, calcium-dependent adenosine triphosphatase and magnesium-dependent adenosine triphosphatase, and glycoproteins such as hexose, hexosamine, fucose and sialic acid in isoproterenol (ISO)-induced myocardial infarction (MI) in rats. Male albino Wistar rats were pretreated with morin (20, 40 and 80 mg/kg) daily for a period of 30 days. After the treatment period, ISO (85 mg/kg) was subcutaneously injected into the rats at an interval of 24 h for 2 days. ISO-induced rats showed significantly ($P < 0.05$) increased activities of cardiac marker enzymes in serum and decreased activities in the heart, and increased activities of calcium-dependent adenosine triphosphatase and magnesium-dependent adenosine triphosphatase in the heart, and the activity of sodium potassium-dependent adenosine triphosphatase decreased in the heart. ISO induction also showed a significant increase in the levels of glycoproteins in serum and the heart. Pretreatment with morin (40 mg/kg) daily for a period of 30 days exhibited significant ($P < 0.05$) effects and altered these biochemical parameters positively compared to the other two doses. Thus, our study shows that morin has a protective role in ISO-induced MI in rats. The observed effects might be due to the free radical-scavenging, antioxidant and membrane-stabilising properties of morin.

K. S. Al-Numair · G. Chandramohan (✉) · M. A. Alsaif
Department of Community Health Sciences,
College of Applied Medical Sciences, King Saud University,
P.O. Box 10219, Riyadh 11433, Saudi Arabia
e-mail: gcmohanphd@yahoo.com

Introduction

Cardiovascular diseases (CVD) are the foremost cause of mortality worldwide. Reduction of the mortality rate and the prevention of myocardial infarction (MI) are of utmost importance. MI is the condition of necrosis of the myocardium that occurs as a result of an imbalance between the coronary blood supply and myocardial demand. Isoproterenol (ISO)-induced cardiac necrosis include increased oxygen consumption, insufficient oxygen utilisation, increased calcium overload and accumulation, changes in the myocardial cell metabolism, increased myocardial cAMP levels, and deranged electrolyte milieu, alterations of membrane permeability, intracellular acidosis and increase in lipid peroxides [1].

ISO [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] is a synthetic catecholamine and β-adrenergic agonist, which has been documented to produce severe stress in the myocardium, resulting in MI, if administered in supramaximal doses [2]. It produces myocardial necrosis which caused cardiac dysfunction and increased lipid peroxidation, along with an increase in the level of myocardial lipids and altered activities of the cardiac enzymes and antioxidants. The pathophysiological and morphological aberrations produced in the heart of this myocardial necrotic rat model are comparable with those taking place in human MI [2]. Among the various mechanisms proposed to explain the ISO-induced cardiotoxicity, the generation of highly cytotoxic free radicals through the auto-oxidation of catecholamines

has been implicated as one of the important causative factors.

Flavonoids are ubiquitous compounds, occurring in various plants, such as tea, herbs, citrus fruits and red wine, and many of them have been shown to be strong free radical scavengers and antioxidants. Several epidemiological studies have supported the hypothesis that the antioxidant actions of flavonoids may reduce the risk of developing CVD [3]. Morin (3,5,7,2′,4′-pentahydroxyflavone; a yellowish pigment) is a bioflavonoid constituent of many herbs and fruits. Bioflavonoids are used as herbal medicines and exhibit various biological activities, including antioxidation, cytoprotection, antimutagenesis and anti-inflammation [4]. 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 [5, 6]. Moreover, morin acts as a chemopreventive agent against oral carcinogenesis in vitro and in vivo [7]. The structure of morin is depicted in Fig. 1.

To date, the effect of morin on cardiac markers, membrane-bound enzymes and glycoproteins in ISO-induced MI rats have not been carried out. These facts motivate us to study the effect of morin on cardiac marker enzymes such as aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and creatine kinase-MB, and membrane-bound enzymes such as sodium potassium-dependent adenosine triphosphatase (Na^+/K^+ ATPase), calcium-dependent adenosine triphosphatase (Ca^{2+} ATPase) and magnesium-dependent adenosine triphosphatase (Mg^{2+} ATPase), and glycoproteins such as hexose, hexosamine, fucose and sialic acid in ISO-induced MI in rats.

Materials and methods

Experimental animals

Male albino rats of the 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 ($25 \pm 1°C$) 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.

Drugs and chemicals

ISO 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 experimental myocardial infarction

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

Experimental design

The animals were randomly divided into six groups of six animals each—Group 1: control rats; Groups 2: normal rats treated with morin (80 mg/kg BW); Group 3: ISO control rats (85 mg/kg BW); Groups 4, 5 and 6: rats pretreated with morin (20, 40 and 80 mg/kg, respectively) 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 (85 mg/kg, s.c.) on the 29th and 30th days in normal saline [8].

A pilot study was conducted with three different doses of morin (20, 40 and 80 mg/kg) in order to determine the dose-dependent effect in ISO-treated rats. It was observed that morin pretreatment at doses of 40 mg/kg significantly ($P < 0.05$) decreased the levels of CK, CK-MB, LDH and AST in serum and increased these levels except for CK-MB in the hearts of ISO-induced rats after 30 days of experimental study compared to that of the other two doses. Hence, doses of 40 mg were chosen for our further study.

After the last treatment, all of 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 homogenised 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 marker enzymes

The activity of CK was assayed in serum and heart tissue by the method of Okinaka et al. [9]. About 0.75 ml of double-distilled water, 0.05 ml of sample, 0.1 ml of ATP, 0.1 ml of
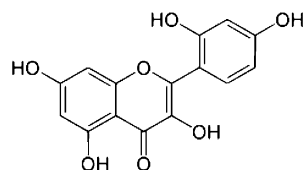
magnesium–cystine reagent and 0.1 ml of creatine (240 mM) contained in tubes were incubated at 37°C for 20 min. The tubes were centrifuged and the supernatant was used for the estimation of phosphorus by the Fiske and Subbarow method [10]. About 1.0 ml of the supernatant was made up to 4.0 ml with distilled water and added to 1.0 ml of 2.5% ammonium molybdate. This was incubated at room temperature for 10 min and 0.4 ml of l-amino-2-naphthol-4-sulphonic acid (ANSA) was added. The colour developed was read spectrophotometrically at 640 nm after 20 min. The activity of LDH and the activities of AST were assayed in serum and the heart tissue using commercial kits purchased from Qualigens Diagnostics (Mumbai, India). CK-MB activity was assayed in serum using an Agappe Diagnostics (Kerala, India) commercial kit.

Assay of glycoproteins

The tissue samples were defatted prior to estimation. A weighed amount of defatted tissue was suspended in 3.0 ml of 2 N HCl and heated at 90°C for 4 h. The sample was cooled and neutralised with 3.0 ml of 2 N NaOH. Aliquots from this were used for the estimation of hexose, hexosamine, fucose and sialic acid.

Protein-bound hexose was estimated by the method of Dubois and Gilles [11]. To 0.1 ml of sample, 5.0 ml of 95% ethanol was added, mixed and then centrifuged. The precipitate was dissolved in 1.0 ml of 0.1 N NaOH. To all of the tubes, 8.5 ml of orcinol–sulphuric acid reagent was added and kept in a water bath at 90°C for 15 min. The tubes were cooled in tap water and the colour developed was read at 540 nm.

Protein-bound hexosamine was estimated according to the method of Wagner [12]. To 1.0 ml of sample was added 2.5 ml of 3 N HCl and kept for 6 h in a boiling water bath and then neutralised with 6 N NaOH. To 0.8 ml of the neutralised sample was added 0.6 ml of acetyl acetone reagent. Then, the tubes were heated in a boiling water bath for 30 min. After cooling, 2.0 ml of Ehrlich's reagent was added and mixed well. The intensity of the colour developed was read at 540 nm.

Fucose was estimated by the method of Dische and Shettles [13]. To 0.5 ml of sample, 4.5 ml of H_2SO_4 was added and heated in a boiling water bath for 3 min, cooled and 0.1 mL of cysteine reagent was added. After 75 min in the dark, the absorbance was read at 393 and 430 nm. The fucose content was calculated from the difference in the readings obtained at 393 and 430 nm, and then the values were subtracted in order to obtain values excluding cysteine.

Sialic acid was estimated by the method of Warren [14]. To 0.5 ml of sample, 0.2 ml of distilled water and 0.25 ml of periodic acid were added and incubated at 37°C for 30 min. To this, 0.2 ml of sodium meta arsenate and 2.0 ml of thiobarbituric acid (TBA) were added and heated in a boiling water bath for 6 min, cooled and 5.0 ml of acidified butanol was added. The absorbance was read at 540 nm.

Assay of membrane-bound enzymes

The activity of Na^+/K^+ ATPase was assayed according to the procedure of Bonting [15]. The incubation mixture contained 1.0 ml of buffer, 0.2 ml of magnesium sulphate, 0.2 ml of potassium chloride, 0.2 ml of sodium chloride, 0.2 ml of EDTA, 0.2 ml of ATP and 0.2 ml of tissue homogenate. The contents were incubated at 37°C for 15 min. A 1.0-ml quantity of ice-cold 10% trichloroacetic acid (TCA) was added at the end of 15 min to arrest the reaction. The tubes were centrifuged at $3,000 \times g$ for 10 min. The amount of phosphorus liberated was estimated as described by Fiske and Subbarow [10]. A 1.0-ml quantity of the supernatant was made up to 4.0 ml with distilled water and 1.0 ml of 2.5% ammonium molybdate was added. This was incubated at room temperature for 10 min and 0.4 ml of amino-naphthol sulphonic acid was added. The colour developed was read spectrophotometrically at 640 nm after 20 min.

The activity of Ca^{2+} ATPase was assayed according to the method of Hjertén and Pan [16]. The incubation mixture contained 0.1 ml of buffer, 0.1 ml of calcium chloride, 0.1 ml of ATP, 0.1 ml of distilled water and 0.1 ml of tissue homogenate. The contents were incubated at 37°C for 15 min. The reaction was then arrested by the addition of 0.5 ml of ice-cold 10% TCA. The contents were centrifuged at 4,000 rpm for 5 min. The amount of phosphorus liberated was estimated according to the method of Fiske and Subbarow [10].

The activity of Mg^{2+} ATPase was assayed according to the method of Ohnishi et al. [17]. The incubation mixture contained 0.1 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of ATP, 0.1 ml of distilled water and 0.1 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 15 min. The reaction was arrested by the addition of 0.5 ml of ice-cold 10% TCA. The tubes were centrifuged at $3,000 \times g$ for 10 min. The amount of phosphorous liberated was estimated by the method of Fiske and Subbarow [10].

Statistical analysis

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

Results

Effect of morin on cardiac marker enzymes

Tables 1 and 2 show the effect of morin on the activities of AST, LDH CK and CK-MB in serum and heart in normal and ISO-induced rats. Rats induced with ISO showed a significant ($P < 0.05$) increase in the activities of these enzymes in serum and decreased in the heart except CK-MB when compared to normal control rats. Pretreatment with morin (20, 40 and 80 mg/kg, respectively) significantly ($P < 0.05$) reversed these changes towards normalcy in ISO-induced rats when compared to ISO-alone-induced rats. Since morin at a dose of 40 mg/kg body weight was significantly effective when compared to the other two doses, the 40-mg/kg dose of morin was taken for further biochemical studies.

Effect of morin on membrane-bound enzymes

Table 3 shows that the activity of Na$^+$, K$^+$ ATPase was decreased significantly ($P < 0.05$) and that the activities of Ca^{2+} and Mg^{2+} ATPases were increased significantly ($P < 0.05$) in the heart of ISO-induced rats when compared

to normal control rats. Morin (40 mg/kg) pretreatment of ISO-induced rats increased significantly ($P < 0.05$) the activity of Na$^+$, K$^+$ ATPase and decreased significantly ($P < 0.05$) the activities of Ca^{2+} and Mg^{2+} ATPases in the heart when compared to ISO-induced rats.

Effect of morin on glycoproteins

The levels of glycoproteins such as hexose, hexosamine, fucose and sialic acid in the serum and heart of normal and ISO-induced rats is shown in Tables 4 and 5. Significantly ($P < 0.05$) increased levels of glycoproteins were observed in the serum and heart of ISO-induced rats in comparison with normal control rats. Pretreatment with morin (40 mg/kg) of ISO-induced rats decreased significantly ($P < 0.05$) these glycoproteins in the serum (Table 4) and heart (Table 5) when compared to ISO-induced rats.

Discussion

When myocardial cells are damaged or destroyed due to the deficiency of the oxygen supply or glucose, the cell membrane becomes permeable or may rupture, which

Table 1 Effect of morin on aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and CK-MB in the serum of control and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

Groups	AST (IU/L)	LDH (IU/L)	CK (IU/L)	CK-MB (IU/L)
Control	76.3 ± 5.24^a	213 ± 15.4^a	153 ± 10.6^a	93.0 ± 6.87^a
Control + morin (80 mg/kg BW)	73.5 ± 5.63^a	199 ± 14.6^a	143 ± 10.1^a	94.0 ± 6.51^a
ISO (85 mg/kg BW)	155 ± 10.8^b	395 ± 10.2^b	403 ± 11^b	382 ± 11.2^b
Morin (20 mg/kg BW) + ISO	118 ± 3.67^c	314 ± 15.4^c	233 ± 7.44^c	244 ± 8.85^c
Morin (40 mg/kg BW) + ISO	94.0 ± 3.9^d	259 ± 6.76^d	189 ± 7.47^d	150 ± 8.24^d
Morin (80 mg/kg BW) + ISO	98.3 ± 5.19^d	269 ± 14.7^d	214 ± 11.4^e	157 ± 8.87^d

Values are expressed as mean $\pm$ standard deviation (SD) for six rats in each group

Values not sharing a common superscript differ significantly at $P < 0.05$ (Duncan's multiple range test [DMRT])

AST units nmol of pyruvate liberated/min/mg protein, *LDH units* nmol of pyruvate liberated/min/mg protein, *CK units* μmol of phosphorus liberated/min/mg protein

Table 2 Effect of morin on AST, LDH and CK in the heart of control and ISO-induced MI in rats

Groups	AST	LDH	CK
Control	40.2 ± 2.93^a	120 ± 9.24^a	12.3 ± 0.87^a
Control + morin (80 mg/kg BW)	40.6 ± 2.89^a	123 ± 9.63^a	12.3 ± 0.85^a
ISO (85 mg/kg BW)	28.5 ± 2.31^b	87.5 ± 6.12^b	6.90 ± 0.59^b
Morin (20 mg/kg BW) + ISO	30.9 ± 2.67^c	92.3 ± 7.35^c	7.80 ± 0.67^c
Morin (40 mg/kg BW) + ISO	36.4 ± 2.90^d	114 ± 8.64^d	10.3 ± 0.78^d
Morin (80 mg/kg BW) + ISO	32.2 ± 2.96^e	104 ± 8.37^e	9.17 ± 0.82^e

Values are expressed as mean $\pm$ SD for six rats in each group

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

AST units nmol of pyruvate liberated/min/mg protein, *LDH units* nmol of pyruvate liberated/min/mg protein, *CK units* μmol of phosphorus liberated/min/mg protein

Table 3 Effect of morin on sodium potassium-dependent adenosine triphosphatase (ATPase), calcium-dependent ATPase and magnesium-dependent ATPase in the heart of control and ISO-induced MI in rats

Groups	Sodium potassium-dependent ATPase (μmol of pyruvate liberated/min/mg protein)	Calcium-dependent ATPase (μmol of pyruvate liberated/min/mg protein)	Magnesium-dependent ATPase (μmol of pyruvate liberated/min/mg protein)
Control	0.38 ± 0.03^a	1.05 ± 0.08^a	5.05 ± 0.42^a
Control + morin (40 mg/kg/day)	0.36 ± 0.03^a	1.01 ± 0.05^a	4.93 ± 0.28^a
ISO (85 mg/kg/day)	0.20 ± 0.03^b	2.15 ± 0.19^b	7.23 ± 0.50^b
Morin (40 mg/kg/day) + ISO	0.32 ± 0.02^c	1.20 ± 0.08^c	5.37 ± 0.29^c

Values are mean ± SD for six rats

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

Table 4 Effect of morin on the levels of glycoproteins in the serum of control and ISO-induced MI in rats

Groups	Hexose (mg/dl)	Hexosamine (mg/dl)	Fucose (mg/dl)	Sialic acid (mg/dl)
Control	124 ± 9.18^a	18.5 ± 1.17^a	20.2 ± 1.49^a	27.3 ± 1.85^a
Control + morin (40 mg/kg/day)	123 ± 9.14^a	18.1 ± 1.15^a	19.3 ± 1.35^a	26.6 ± 1.70^a
ISO (85 mg/kg/day)	199 ± 14.6^b	26.5 ± 1.60^b	32.3 ± 2.18^b	36.5 ± 2.57^b
Morin (40 mg/kg/day) + ISO	135 ± 9.36^c	22.3 ± 0.98^c	24.5 ± 1.62^c	30.2 ± 2.62^c

Values are mean ± SD for six rats

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

Table 5 Effect of morin on the levels of glycoproteins in the heart of control and ISO-induced MI in rats

Groups	Hexose (mg/g defatted tissue)	Hexosamine (mg/g defatted tissue)	Fucose (mg/g defatted tissue)	Sialic acid (mg/g defatted tissue)
Control	135 ± 9.18^a	4.20 ± 0.28^a	23.3 ± 1.14^a	35.3 ± 1.68^a
Control + morin (40 mg/kg/day)	136 ± 9.25^a	4.01 ± 0.27^a	23.3 ± 1.17^a	34.9 ± 1.73^a
ISO (85 mg/kg/day)	171 ± 12.34^b	6.70 ± 0.43^b	29.9 ± 1.55^b	46.6 ± 2.13^b
Morin (40 mg/kg/day) + ISO	142 ± 11.78^c	4.56 ± 0.30^c	25.4 ± 1.35^c	41.5 ± 2.10^c

Values are mean ± SD for six rats

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

results in the leakage of enzymes. We have observed an increase in the activities of AST, LDH, CK and CK-MB in serum and decreased activities in the heart except for CK-MB in the myocardium of ISO-induced rats. The release of cellular enzymes reflects the alterations in the plasma membrane integrity and/or permeability as a response to β-adrenergic stimulation. This might be due to the damage caused to the sarcolemma by the β-agonist that has rendered it leaky. ISO induction produces free radicals via the β-adrenoceptor mechanism, which affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis [18]. Pretreatment with morin decreased the activities of these enzymes in serum and increased the activities of these enzymes in the heart of ISO-induced rats. This could be due to the

protective effect of morin on the myocardium, reducing the cardiac damage, thereby, restricting the leakage of these enzymes. Assay of the activity of CK-MB in serum is an important diagnosis because of the marked abundance of this enzyme and virtual absence from most other tissues and its consequent sensitivity. The CK-MB isoenzyme activity is useful not only as an index of early diagnosis of MI, but any type of myocardial injury. The increased activity of serum CK-MB observed in ISO-induced rats might be due to cardiac damage induced by ISO [19]. Pretreatment with morin decreased the activity of serum CK-MB in ISO-induced rats. In this context, Kang et al. [20] reported that morin, a flavonoid, exhibited a significant beneficial effect on blood pressure, lipid profiles, and serum insulin and glucose in high-fat-induced hypertensive

rats. A report by Subash and Subramanian [21] shows that the morin (30 mg/kg body weight) by oral administration offers protection against hyperammonaemia by means of reducing blood ammonia, oxidative stress and enhancing the antioxidant status in ammonium chloride-induced (100 mg/kg body weight; i.p.) hyperammonaemic rats. Also, Wu et al. [5] reported that morin acts as a potent antioxidant. This effect might be due to the free radical-scavenging and antioxidant property of morin [5]. Morin is a moderately potent inhibitor of xanthine oxidase (XO) [22]; 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.

Membrane-bound enzymes play a significant role in maintaining ion levels within the myocytes. Any alteration in the properties of these enzymes is known to affect the function of the heart. Failure of the cell membrane to maintain normal transmembrane ionic distribution through ion pumps is considered to be a major event in the pathogenesis of ischaemia and arrhythmia. In this study, we observed decreased activity of Na^+/K^+ ATPase and increased activities of Ca^{2+} and Mg^{2+} ATPase in ISO-induced rats. The inactivation of Na^+/K^+ ATPase could be due to enhanced lipid peroxidation by free radicals on ISO induction, since Na^+/K^+ ATPase is an 'SH' group-containing enzyme and is lipid-dependent [23]. Enhanced Ca^{2+} ATPase in ISO-induced rats is due to adenylate cyclase activation by ISO. Calcium overload in the myocardial cells during ischaemia activate the Ca^{2+}-dependent ATPase of the membrane, depleting high-energy phosphate stores, thereby, indirectly inhibiting Na^+ and K^+ transport and the inactivation of Na^+/K^+ ATPase [23]. Pretreatment with morin increased the activity of Na^+/K^+ ATPase and decreased the activities of Ca^{2+} and Mg^{2+} ATPases in ISO-induced rats. This could be due to the ability of morin to protect the 'SH' groups from oxidative damage through the inhibition of the peroxidation of membrane lipids. This effect might be due to the membrane-stabilising action of morin.

Glycoproteins are involved in the myocardial necrosis and repair. Increased levels of hexose, hexosamine, fucose and sialic acid were observed in ISO-induced rats. Glycoproteins levels are increased significantly in CVD. The increased serum levels of glycoprotein components might be due to the secretion from cell membrane glycoconjugates into the circulation [24]. Increase in the levels of glycoproteins in ISO-treated rats may also be due to the increased deposition of macromolecular components, which is a physiological adjustment to the pathological process. The levels of serum and heart glycoproteins increased in ISO-induced MI rats [25]. Pretreatment with morin decreased the levels of glycoproteins in serum and heart compared to ISO rats.

The observed effects of morin might be due to its free radical-scavenging, antioxidant and membrane-stabilising properties. Our study shows that morin exhibits a cardioprotective effect in MI rats by reverting membrane-bound enzymes and glycoprotein levels to near-normal. A previous report shows the preventive effect of s-allylcysteine and α-tocopherol on membrane-bound enzymes and glycoproteins in normal and ISO-induced cardiac toxicity in male Wistar rats [26]. Karthik Kumar et al. [27] reported that morin lessens the glycoprotein components in experimental colon carcinogenesis. This effect might be due to the free radical-scavenging, antioxidant and membrane-stabilising effects of morin. In this context, Sreedharan et al. [28] reported 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. Morin contains five hydroxyl groups in the aromatic ring system. The $2',4'$ hydroxyl configuration in the B ring requires for scavenging free radicals. Thus, the presence of hydroxyl groups at positions $2',4'$ may be the cause for the cardioprotective effect of morin. As a result, our study further supports the cardioprotective effect of morin in ISO-induced rats. Further studies are underway in order to elucidate the molecular mechanisms involved in proving morin's efficacy as a cardioprotective agent.

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