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

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Introduction

- Myocardial infarction is the condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand.
- Isoproterenol (ISO)-induced cardiac necrosis include increased oxygen consumption, insufficient oxygen utilization, increased calcium overload and accumulation, changes in myocardial cell metabolism, increased myocardial cAMP levels, and deranged electrolyte milieu, alterations of membrane permeability, intracellular acidosis and increase in lipid peroxides.
- 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.
- Morin (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.

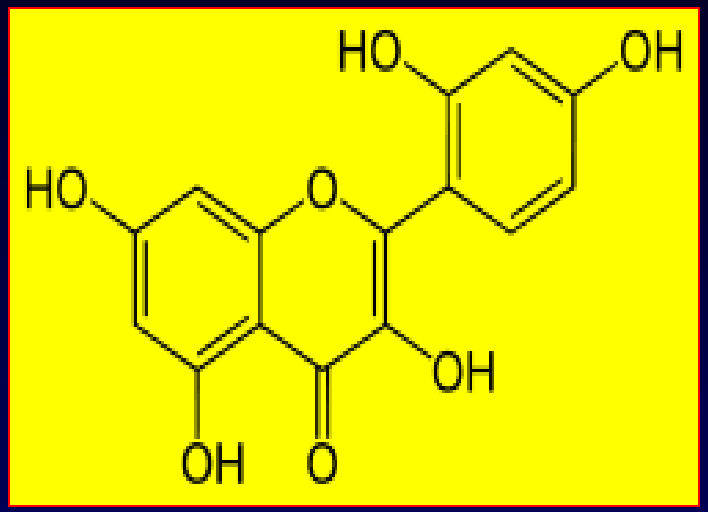
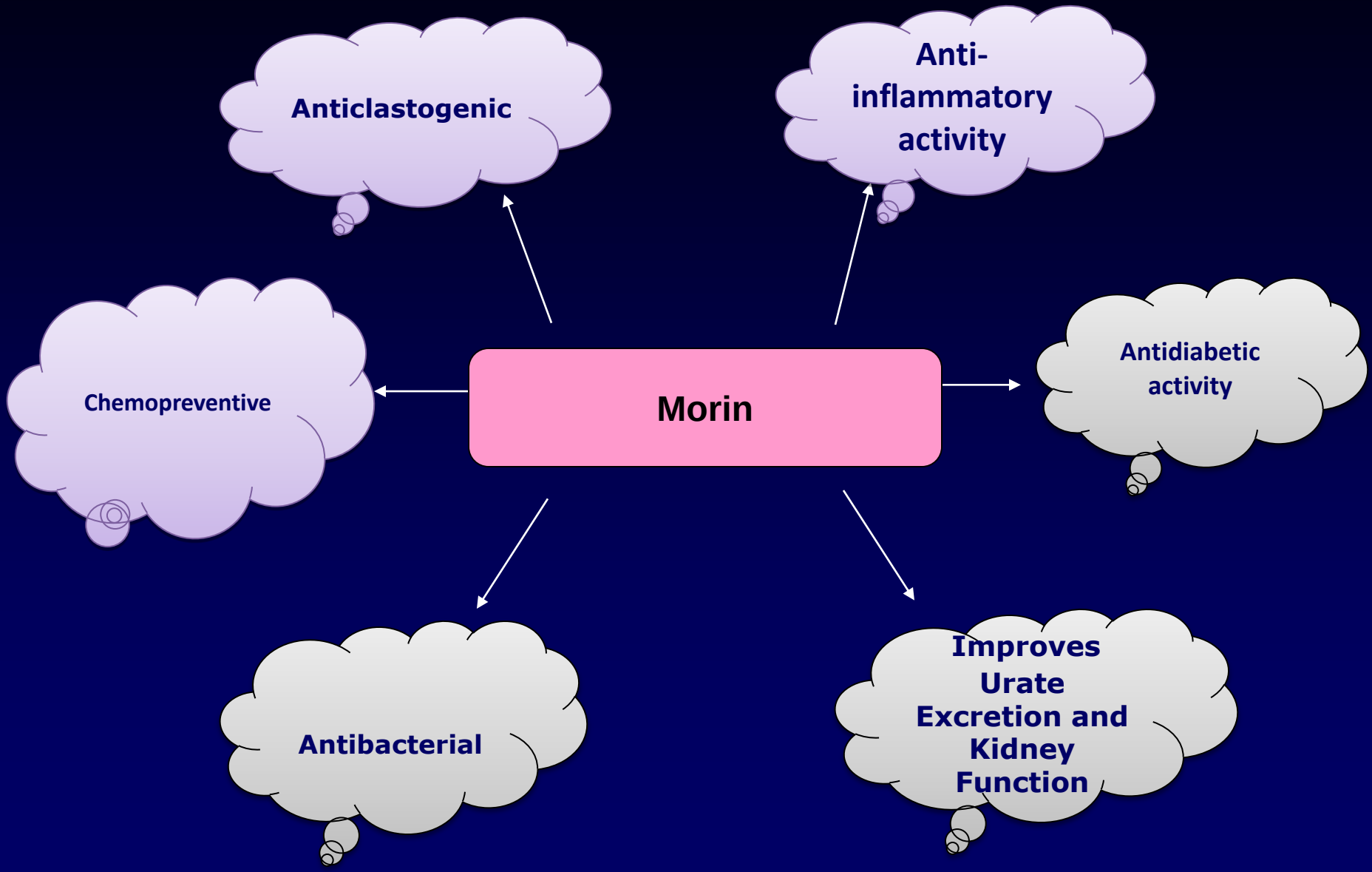


Fig 1. Structure of Morin

Pharmacological activities of morin



OBJECTIVES

- To study the effect of morin on cardia marker enzymes such as aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), and creatine kinase-MB.
- To study the membrane bound enzymes such as sodium potassium-dependent adenosine triphosphatase (Na+/K+ ATPase), calcium dependent adenosine triphosphatase (Ca2+ ATPase) and magnesium-dependent adenosine triphosphatase (Mg2+ATPase)
- To study the glycoproteins such as hexose, hexosamine, fucose and sialic acid in ISO-induced MI in rats.

Material and Methods

Animals

- Male albino Wistar rats (140-160g)

Induction of experimental diabetes

- Myocardial ischemia was induced by subcutaneous injection (s.c.) of isoproterenol hydrochloride (85mg/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)

- 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.

Biochemical estimations	Methods/Techniques
➢*Aspartate transaminase (AST)*	*Commerical Kit*
➢*Lactate dehydrogenase (LDH)*	*Commerical Kit*
➢*Creatine kinase (CK)*	*Okinaka (1961)*
➢*Creatine kinase (CK)-MB*	*Commerical Kit*
➢ *Na+/K+ ATPase*	*Bonting (1970)*
➢*Ca2+ ATPase*	*Hjerten and Pan(1983)*
➢*Mg2 + ATPase*	*Ohnishi et al (1982)*
➢*Hexose*	*Dubois and Gilles (1956)*
➢*Hexosamine*	*Wagner (1979)*
➢*Fucose*	*Dische and Shettles (1948)*
➢*Sialic Acid*	*Warren (1959)*

Statistical analysis - One way ANOVA followed by DMRT ($p<0.05$)

RESULTS AND DISCUSSION

Table 1 Effect of morin on AST, LDH, CK and CK-MB in the serum of control and 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 means ± S.D. for six rats in each group.

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

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

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

AST unit: nmol of pyruvate liberated min/mg/ protein; LDH unit: nmol of pyruvate liberated min/mg/protein

CK units: µmol of phosphorus liberated min/mg/protein.

Values are expressed as means ± S.D. for six rats in each group.

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

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

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

Values are expressed as means ± S.D. for six rats in each group.

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

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

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

Values are expressed as means ± S.D. for six rats in each group.

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 myocardial infarction (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 ± 9.18a	4.20 ± 0.28a	23.3 ± 1.14a	35.3 ± 1.68a
Control + Morin (40 mg/kg/d)	136 ± 9.25a	4.01 ± 0.27a	23.3 ± 1.17a	34.9 ± 1.73a
ISO (85 mg/kg/d)	171 ± 12.34b	6.70 ± 0.43b	29.9 ± 1.55b	46.6 ± 2.13b
Morin (40 mg/kg/d) + ISO	142 ± 11.78c	4.56 ± 0.30c	25.4 ± 1.35c	41.5 ± 2.10c

Values are expressed as means ± S.D. for six rats in each group.

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

- ✓ When myocardial cells are damaged or destroyed due to the deficiency of oxygen supply or glucose, the cell membrane becomes permeable or may rupture and results in the leakage of enzymes.
- ✓ In our study, activities of AST, LDH, CK, CK-MB where increased in serum and AST, LDH, CK where decreased in heart of the myocardium of ISO-induced rats.
- ✓ Pretreatment with morin reversed these changes towards normalcy due to the protective effect of morin on the myocardium, reducing the cardiac damage thereby restricting the leakage of these enzymes and also these preventing changes might be due to the free radical scavenging and antioxidant property of morin.

- ✓ **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 heart.Failure of the cell membrane to maintain normal trans membrane ionic distribution through ion pumps is considered to be a major event in pathogenesis of ischemia and arrhythmia.**
- ✓ **In this study, we observed decreased activity of Na+/K+ ATPase and increased activities of Ca2+ and Mg2+ ATPase in ISO-induced rats.**
- ✓ **Pretreatment with morin increased the activity of Na+/K+ ATPase and decreased the activities of Ca2+ and Mg2+ ATPases in ISO-induced rats. This could be due to the ability of morin to protect the 'SH' groups from the oxidative damage through the inhibition of peroxidation of membrane lipids.**

- ✓ **Glycoproteins are involved in the myocardial necrosis and repair.**
- ✓ **Increased levels of hexose, hexosamine, fucose and sialic acid were observed in ISO-induced rats.**
- ✓ **Pre-treatment with Morin decreased levels of glycoproteins in serum and heart against ISO- rats which might be due to the Morin having free radical-scavenging, antioxidant and membrane-stabilizing properties.**
- ✓ **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 cardio protective effect of Morin.**

CONCLUSION

- Further studies are underway to elucidate the molecular mechanisms involved to prove morin's efficacy as an cardio protective agent.

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Thanks' for your kind attention!!!!!!

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