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Protective role of morin on some parameters related to oxidative stress and metabolic syndrome induced by acrylamide in male rats

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ABSTRACT

The objectives of this study were to explore the effects of *morin* on some physiological parameter including lipid profile, antioxidant status, and pro-inflammatory biomarkers in Acrylamide (ACR) treated rats. Forty adult male rats were selected randomly and equally allocated into four bunches that were G1, G2, G3 and G4, which were treated daily for 45 days with an oral manner as follows: G1: (control group received distilled water). G2: rats in this group were received (25 mg/kg/B. W) of morin. G3: rats of this group were received (1 mg/kg/B. W) of acrylamide in drinking water G4: rats in this group were received (25 mg/kg /B. W) of morin and Acrylamide (1 mg/kg /B. W). Fasting blood samples were collected by cardiac puncture technique at the end of the experiment. Blood was drawn by cardiac puncture technique for measuring the following parameters: total serum cholesterol (TC), (TAG) HDL-C, uric acid, Malondialdehyde (MDA) and glutathione (GSH). Sections of the liver were taken for histopathological study. The results of the current study revealed the beneficial effect of morin against deleterious effects of ACR illustrated by its antioxidant, anti-inflammatory effect. In conclusion, morin alleviates the determined effect of ACR correlated with their pro-oxidative and pro-inflammatory effects.

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INTRODUCTION

Metabolic syndrome or android obesity is characterized by a group of plaid of interconnected physiological, biochemical, clinical and metabolic variables that expands the danger of cardiovascular disease (CVD), type 2 diabetes mellitus (Sookoian and pirola, 2011) and renal diseases (Sanchez-Lozada *et al., 2008*) directly. Met S is a condition of chronic low-grade inflammation resulted from complex interactions of hereditary with the environmental factors. Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, hereditary susceptibility, high blood pressure, hypercoagulability condition and chronic stress are the main criteria of disorders (Spolidoro, 2013 and Kaur, 2014). 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 antioxidant cytoprotection, antimutagenesis and anti-inflammation. 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). Moreover, morin acts as a chemopreventive agent against oral carcinogenesis *in vitro* and *in vivo* (Brown *et al.,* 2003). Acrylamide (ACR), a small molecule, characterized by its high reactivity. It has been utilized worldwide through the last five decades for the manufacture of polyacrylamide

polymers that were primarily used as flocculants for clarifying drinking water and food packing. Gel electrophoresis, wastewater treatment, the formation of groundwater, papermaking, and the manufacture of dyes are the significant uses of polyacrylamide and ACR (Lineback *et al.,* 2012). Heightened concerns about exposure to ACR arose in 2002 when it was discovered that it forms when certain foods exposed to a high temperature causing severe toxicity (Eriksson, 2005). Acrylamide is produced in starchy foods those are baked, roasted or fried at high temperature (Rommens *et al.,* 2008). Bread, crisps, coffee and fried potato are the most contaminated food with ACR. In addition to potato salting biscuits contain a considerable amount of ACR (Sirot *et al.,* 2012). The primary source of human exposure to Acrylamide is occupational other sources include food, drinking water and smoking, Acrylamide is neurotoxic to both experimental animal and human (Zhang *et al.,* 2011) and has mutagenic and carcinogen effects (Bongers *et al.,* 2012 and Dewoskin *et al.,* 2013). Acrylamide also reported causing hepatotoxicity (AL-Mosaibih, 2013), DNA damage (Besaratinia and Pfeifer, 2007) and reproductive toxicity (Nixon *et al.,* 2014).

MATERIALS AND METHODS

Experimental Design

Forty (40) adult male rats were randomly selected and equally divided into four groups that were (G1, G2, G3 and G4). They were treated orally for 45 days continuous as following: G1: (control group received distilled water). G2: rats in this group were received (25 mg/kg /B.W) of morin. G3: rats of this group were received (1 mg/kg /B.W) of acrylamide in drinking water G4: rats in this group were received (25mg/kg /B.W) of morin and Acrylamide (1 mg/kg /B.W).

Blood Sampling

During fasting, the blood samples were collected at (0, 7 weeks) of the experiment. Blood was drawn by cardiac puncture technique from rats anaesthetised by intramuscular injection of xylazine (40 mg/kg B.W.) and ketamine (90 mg/kg B.W) using a disposable syringe of needles 3 cm. Blood samples were kept in tubes and followed by centrifugation for 15 minutes at 3000 rpm, then sera were isolated and frozen at -20˚C until analysis of the following parameters:

Serum biochemical parameters

- Determination of serum total cholesterol (TC) concentration (mg /dl).
- Determination of serum triacylglycerol (TAG) concentration (mg/dl).
- Determination of serum high density lipoprotein-cholesterol (HDL-C) concentration (mg/dl).
- Determination of serum uric acid, concentration (mg/dl).
- Determination of serum reduced glutathione (GSH) concentration (µml/l).
- Determination of serum MDA concentration (µml/l).

Histopathological study

For histological sections, rats were anaesthetised, sacrificed by the withdrawal of blood from heart instantly. After scarification, the liver was excised blotted opened longitudinally and preserved in 10% neutral formalin puffer solution till the planning of histological section. Several tissue sections were prepared from the liver of experimental rats and stained with Hematoxylin-Eosin, according to methods described by Lee and Luna (1968).

Statistical Analysis

Statistical analysis of data was performed according to for Two-Way Analysis of Variance (ANOVA) utilising a significant level of (P<0.05).

RESULTS

Effects of Morin on serum total cholesterol (TC) concentration (mg/dl) in Acryl amide treated rats

Table (1) pointed to the values of serum total cholesterol concentration in rats exposed to morin and Acrylamide for 45 days. The results showed significant (P<0.05) increase in serum total cholesterol concentration in rats received 1mg/kg B.W ACR for 45 days (G2) groups compared to the observed values in other treated groups and control. While continues gavage of 25 mg/kg B.W of morin for 45 days (G3group) caused significant (P<0.05) decrease in serum TC concentration comparing to the G2 groups and in the combination group shown significant with acrylamide group.

Effects of Morinon serum triacylglycerol (TAG) concentration (mg/dl) in Acryl amide treated rats

A statistical analysis of the mean values of serum TAG concentration in rats exposed to morin and acrylamide for 45 days showed a significant increase (P<0.05) in this parameter was observed in G2 (ACR) treated, comparing to the other groups. On other hands, oral intubation of morin caused significantly (P<0.05) decrease in serum TAG concentration comparing to ACR treated group indicating the hypolipidemic effect of morin and in the combination group shown significant with acrylamide group.

Table 1: Effect of Morin on males rats treated with the ACR

No.	Parameters	Control	ACR	Morin	Combination
1	TC	77.39±1.401	128.49 ±2.540	76.55±0.625	99.32± 5.331
2	TAG	152.91±1.463	225.22±2.312	155.32±1.01	190.69± 3.024
3	HDL-C	45.72 ± 0.470	23.46 ±1.485	44.57 ± 1.109	34.57 ± 1.109
4	Uric acid	1.50 ± 0.109	3.41 ± 0.105	1.65 ± 0.01	2.06 ± 0.257
5	GSH	57.8 ± 1.02	47.8 ± 1.98	58.9±1.62	62.00 ± 1.58
6	MDA	1.66±1.383	2.85 ± 1.490	1.84±0.5	2.13 ± 2.034

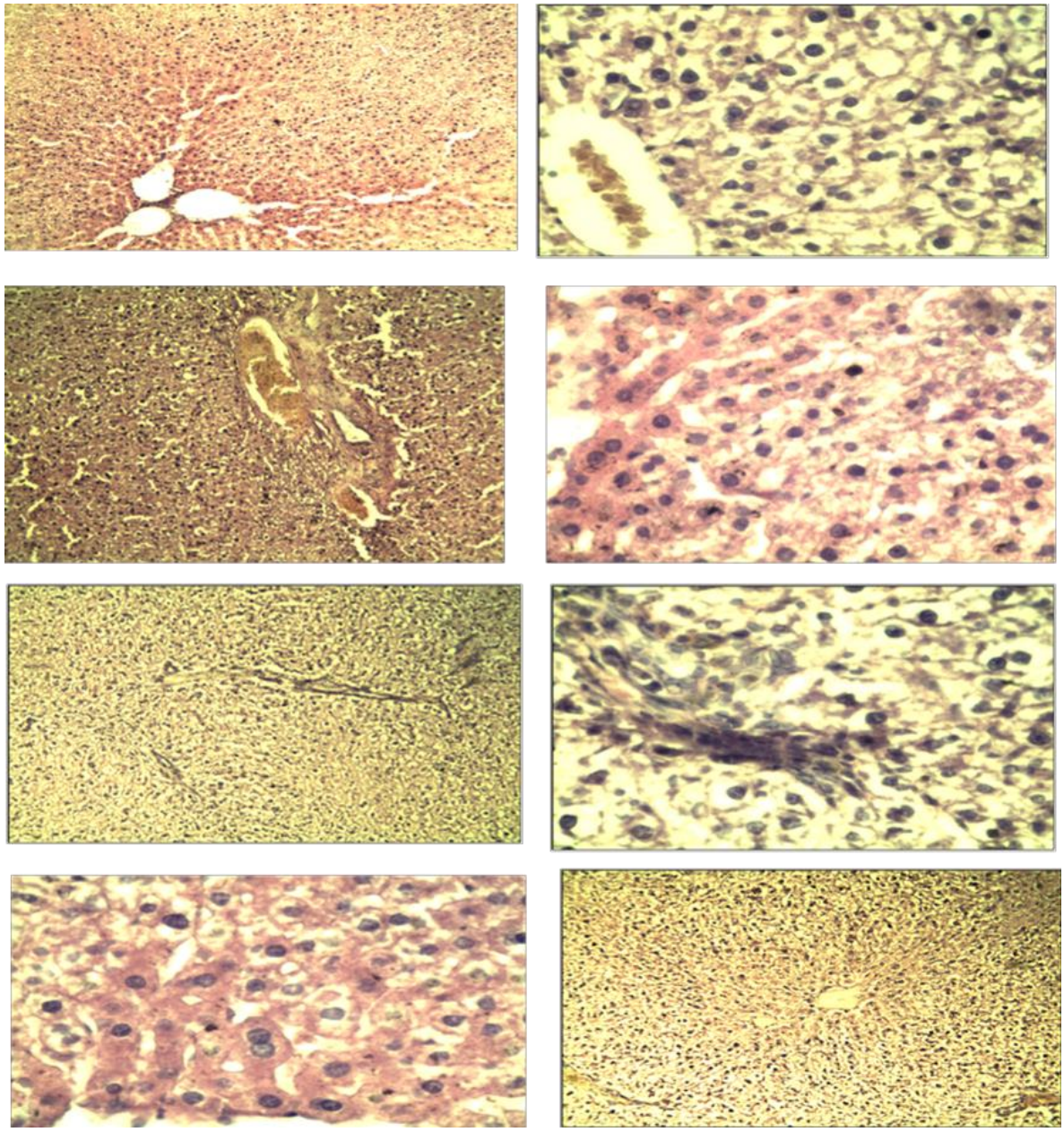

Figure 1: Effect of Morin on males rats treated with the ACR

Effects of Morinon serum high-density lipoprotein HDL-C concentration (mg/dl) in Acryl amide treated rats

Table (1) illustrated the effect of oral intubation of 25 mg/kg B.W of morin for 45 days on serum HDL-concentration of acrylamide treated rats. The result showed that oral intubation of morin (combination group) with acrylamide caused significant (P<0.05) elevation in serum HDL-C concentration compared to the values in ACR (G2) treated groups.

Effects of Morinon serum uric acid concentration (mg/dl) of Acryl amide treated rats

Table (1) illustrated serum uric acid concentration in rats exposed to morin and acrylamide (ACR) for 45 days. The result showed the result appeared a significant increase (P<0.05) in this parameter in

acrylamide (G2) treated group compared to the values in the control group. On the other hand, a significant decrease (P<0.05) in serum uric acid concentration was observed after oral intubations of morin compared to the value in ACR treated groups.

Effects of Morinon serum glutathione (GSH) concentration) µM/L (in Acryl amide treated rats

The mean values of serum glutathione concentration in rats exposed to Acrylamide, and Morin for 45 days are clarified in the table (1). There was a highly significant difference (P<0.05) in the mean values of serum GSH concentration in all experimental groups as compared to each other. Continuous treatment with ACR significant (P<0.05) reduction in this parameter at the end of the experimental (45 days) in G2 treated group compared to the values in G2, G3 and control group indicating peroxidant activity of ACR. Besides oral intubation of morin concurrently with ACR caused significant (P<0.05) elevation in serum GSH concentration in groups G4 and groups G2 compared to the values in ACR treated groups.

Effects of Morin on serum MDA (µM/L) of rats in Acrylamide treated rats

Table (1) indicated the mean value of serum MDA concentration of rats exposed to morin and Acrylamide for 45 days. The results a significant increase (P<0.05) in this parameter was observed in G2 (ACR) treated group compared to other groups indicated the free radical inducing activity of ACR. Besides, a significant decrease (P<0.05) in serum MDA concentration was observed in (G3 and G4) groups after gavage morin concurrently with ACR as compared with G1 group.

Histological finding

In a recent study, attention has been focused on the role of biotransformation of ACR to highly reactive metabolites that initiate cellular toxicity, orally consumed ACR is absorbed into the circulation, then distributed to various organs reacts with DNA, neurons, described the vacuolation of hepatocytes as ballooning degeneration and interpreted it as a kind of cellular defensive mechanism against injurious substances. While in groups (morin & combination) we have shown the effect of morin in the repair of the tissue in the liver. In fig (1,2) liver of rats, Gavage of normal saline for 45 days, shown normal control vein, present of a radial arrangement of hepatocyte and proliferation in liver tissue. While in fig. (3,4) Section of Liver of rat, Gavage of ACR (1 mg/kg B.W.) for 45 days. Congestion and hyper aplasia of the bile duct in liver tissue showed binucleated, degeneration of hepatocyte in liver tissue. In fig (5,6) liver of rats, Gavage of morin (25mg / kg B.W.) for 45 days. Normal control vein, present of radial arrangement of hepatocyte and proliferation in liver tissue and fig. (7,8) Liver of rats, Gavage of morin (2.5g / kg B.W.) one time and Gavage of ACR 1mg/kg B.W.) for 45 days. Clear regeneration of hepatocyte which showed vacuolated and bionuclatid, mild dilation of cytoside in liver tissue, clear regeneration of hepatocyte which showed vacuolated and bionuclatid.

DISCUSSION

Metabolic syndrome or android obesity is a pathophysiological status encapsulated the following five specific criteria: abdominal obesity, high fasting glucose, low serum HDL-C, high serum TAG and elevated blood pressure (Redon *et al.,* 2009). Three of the five signs must be present to report a case of MetS (Spolidoro *et al.,* 2013). Hyperuricemia is frequently encountered in patients with the metabolic syndrome and was a minor criterion for the diagnosis of insulin resistance syndrome, some indicators of oxidative stress markers (MDA, GSSG, and ROS) are elevated in an obese subject, especially in those exhibiting metabolic syndrome (Hajjar and Gotto, 2013). Depending on the result obtained from the current study what was central obesity, elevation in TAG, lowered HDL-C concentration, hyperglycemia, hyperuricemia, elevation in MDA and depression of serum GSH concentration, it can be concluded that ACR can induce metabolic syndrome. Dyslipidemia is generally characterized by lipid abnormalities reflecting perturbations in the biological activity, metabolism and structure of both antiatherogenic HDL- C and atherogenic lipoprotein (LDL) leading to elevation in lipoprotein containing apolipoprotein B (apo B), TAG and low level of HDL-C, The data obtained from this study regarding the effect of ACR on serum lipid profile (significant increase in serum TC and TAG with significant depression in serum HDL-C concentration), who observed a decrease in the levels of previous biomarker (TC and TAG) after trace ACR exposure in rats, which was explained to the decrease in serum insulin level that cause decrease in TAG concentration and cholesterol. On the contrary, the finding of the current study is in agreement with (Allam *et al.,* 2010). A significant increase in serum lipid profile (except HDL-c that was decreased) was observed in rats received 50 mg/kg B.W of ACR for consecutive 5days or received a standard diet containing 50-60 mg/kg B.W of ACR for 35days (Teodor *et al.,* 2011). ACR caused changes (elevation) in TC concentration may be due to an increase in the relative weight of liver since it is regarded as a target for ACR toxicity (Allam *et al.,* 2010). Another explanation could be attributed to the increment in the synthesis of

plasma lipoprotein and high mobilization of lipid from the liver. Rawi *et al*. (2012) indicated that a change in lipid profile might be attributed to liver disorder induced by ACR. That effect seriously, the capacity of the liver to process circulating lipoproteins which was documented in the current study by histopathological changes indicated hepatic damage. IR induced after ACR exposure (Lin *et al.,* 2009) may lead to atherogenic dyslipidemia through suppression of lipolysis in adipocyte attributed to impairment in insulin signalling, elevated levels of free fatty acids (FFA) in the liver that serves as a substrate for the synthesis of TAG leading to hyper triacyglycerieldemia. The FFA also stabilizes apo B (the major apoprotein of VLDL particle) production leading to fatty liver and atherogenic dyslipidemia. Besides, the expected IR after ACR exposure may cause a decline in VLDL clearance, through inhibition of lipoprotein lipase leading to an elevation in TAG concentration. Abdominal obesity, recognized by increased waist circumference, is the first criteria listed to be contributed to metabolic syndrome. Visceral fat appears to be the most detrimental contributor to the development of lipotoxicity in peripheral tissue by adipocytokines secretion. Adipocytokines secretion integrates autocrine and paracrine signal that mediate insulin sensitivity and oxidative stress, the two criteria contribute to ACR toxicity and may claim to be responsible for visceral obesity accompanied ACR (Zhang, 2011). Adiponectin, a protein hormone with inflammatory activity, is negatively correlated with fasting insulin, IR (Khanna and Mali, 2010), visceral obesity. On the other hand, a positive correlation existed between adiponectin and HDL-C level, going in line with this information it can be concluded that IR accompanied ACR treatment may lead to decrease in adiponectin coincide with an increase in visceral obesity as well depression in HDL-C concentration. Chronic hypersecretion of stress mediators, like cortisol in subjects, may lead to accumulation of visceral fats. When glucocorticoids elevated, the activities of enzymes involved in lipogenesis and accelerated and promote lipoprotein secretion, promote differentiation of preadipocyte to adipocyte which could lead to increase body fat mass, such hormonal alterations increasing abdominal obesity. Accordingly, the expected stress induced after ACR exposure may cause an elevation in serum cortisol concentration with the progress of cascade of events leading to visceral obesity. Uric acid is a secondary parameter for assessing the renal function and its one of important criteria of metabolic syndrome. Exposure to ACR caused a significant increase in serum uric acid concentration (Teodor *et al.,* 2011), indicating renal dysfunction. The current data regarding the effect of ACR on serum uric acid concentration (Hyperuricemia) is in accordance with other previous studies (Abd El-Mottaleb and Rashed, 2008). A case of dyslipidemia referred to hypercholesterolemia, elevation in serum TAG and reduction in HDL-C concentration was found to be correlated with hyperuricemia and metabolic syndrome by some investigators. In the current study, serum lipid profile was similarly affected by ACR which may explain its mechanism in hyperuricemia. Besides, a significant elevation in the release reactive oxygen species and the lipid peroxidation, inducing oxidative stress by ACR accompanied by depletion in the antioxidant level of the kidney (Al-Turfan *et al.,* 2011) could impair renal function leading to hyperuricemia. Hyperuricemia observed in the current study was accompanied with moderate histopathological changes in the kidney, attributed to the fact indicating that the kidney is the way for excretion of ACR and its metabolites and hence transient impairment in renal function. Here in the study indicated that exposure to 1 mg/kg B.W of acrylamide in male rats caused a significant decrease in serum GSH concentration indicating a case of oxidative stress. An increase in level of thiobarbituric acid reactive substance (TBRS) a marker of LPO in the serum and many tissue after ACR exposure (Allam *et al.,* 2013), accompanied with reduction in other antioxidant enzymes like GST, SOD (Teodor *et al.,* 2011 and Swamy *et al.,* 2013) and GSH (Mogda *et al.,* 2008), has been regarded as signs of ACR toxicity. Zhang *et al*. (2011) reverted the damaging effect of ACR to antioxidant defense system of the body causing releasing of ROS leading to diminished in GSH concentration. Reduced GSH is regarded as an important cellular antioxidant in mammalian cells (Tong *et al.,* 2004), necessary for GSH reductase activity, such depression in GSH concentration after ACR exposure impaired the ability of the cell to detoxify reactive intermediate such as hydroxyl radical and hydrogen peroxide leading to oxidative stress. The reduction in activity of GR enzyme due to an interaction of ACR with SH group present in the active site of the enzyme, preventing its precipitation in the formation of reduced GSH, could be a possible way for depression in GSH level after exposure. Besides, the oxidation of ACR to the reactive epoxide (glycidamide), lead to its conjugation with GSH, and thus a reduction in GSH concentration. The case of oxidative stress after ACR exposure was also attributed to an elevation in serum peroxynitrite concentration, the most toxic reactive nitrogen species that cause oxidative stress and serious damage in the tissue (Yu *et al.,* 2013). Hyperuricemia detected in the present study could be claimed as a possible mechanism for elevation in peroxynitrite after ACR exposure. Elevation in uric acid-induced oxidative stress manifested by a rise in H2O2, an important ROS in the body, as well elevation in superoxide onion that reacts with NO

forming peroxynitrite. ACR induced hepatotoxicity as the metabolism of it mediated through glutathione conjugation in the hepatic tissue, It can be concluded that enhanced LPO and deterioration of antioxidant defense system resulted from ACR exposure (Yu *et al.,* 2013) may play a significant role in pathogenesis and deleterious histopathological changes accompanied ACR intoxication in different organs. Selenium and curcumin extract reduced hepatotoxicity observed in sodium fluoride-treated mice through the restoration of histopathological changes (AL-Harbi *et al.,* 2014). General is speaking, the antioxidant activity of morin responsible for maintaining cellular integrity and normal body functions could be attributed to selenium renoprotective activity. 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 ACR-induced myocardial infarcted rats. 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. 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. 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. The previous report shows that the morin offers to protect against hyperammonemia by reduced oxidative stress and enhanced antioxidant activity in ammonium chloride-induced hyperammonemia rats (Subhash and Subramanian, 2009). Flavonoid antioxidants function as scavengers of free radicals by rapid donation of the 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 chelating one or more metal ions such as Fe 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.

CONCLUSION

This study demonstrates that morin protected the myocardium against ACR-induced oxidative stress and metabolic syndrome and suggested that these effects could be due to the prevention or inhibition of lipid peroxidative system by its antioxidant effect.

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