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Modulatory Effect of Morin on D-Galactosamine Induced Hepatotoxicity in Rats

L. Sakthi Priya, M. Chitra* and T. Manivasagam

PG and Research Department of Biochemistry, S.T.E.T.Women's College, Mannargudi, Tamilnadu-641 001
Department of Biochemistry and Biotechnology, Annamalai University, Chiambaram

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Abstract: Effect of the morin against D-galactosamine induced heptotoxicity was investigated in male albino wister rats. The parameters studied were liver marker enzymes and bilirubin lipid profile, lipid peroxidation and antioxidant status in experimental animals. It was observed that all these levels were increased in D-galactosamine induced rats in morin treatment. These alterations were reversed by administration of morin. The present study also appraised alteration in the level of collagen and inflammation αSMA. The expression of collagen and inflammation levels is increased in D-galactosamine induced rats, however, regained by morin treatment.

Key Words: Liver toxicity, D-galactosamine, αSMA, lipid peroxidation

Introduction

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver disease. Developing countries like India and others have struggled to manage the impact of hepatitis along with the growing burden of obesity, type II diabetes, hypertension and coronary heart disease [1]. A better understanding of the processes involved in hepatitis has stimulated the search for new drugs, which could limit the drug-induced hepatic injury.

Liver toxicity due to poisons used in experimental model rarely occurs in human beings. It is, therefore, important to use hepatotoxic agents that are more relevant to human beings such as ethyl alcohol and D-galactosamine (D-GalN). Liver damage due to direct action of drugs is associated with D-GalN as the administration induces inflammatory response in liver that resembles the reaction seen in viral hepatitis and in fact D-GalN-induced hepatitis resembles viral hepatitis both biochemically and histologically [2].

Apart from the well documented inhibition of protein synthesis, other mechanisms must be involved in D-GalN hepatotoxicity [3]. In recent years, it has been suggested that reactive oxygen species produced by activated hepatic macrophages might be the primary cause in D-GalN-induced liver damage [4-5]. Sun *et al* [6] reported that D-GalN-induced necrosis is the result of extensive free radical generation reaction or oxidative stress.

Morin is bioflavonoid with antioxidant properties, shows intestinal anti-inflammatory activity in the acute phase of the trinitrobenzene sulphonic acid model of rat colitis [6a]. The chemopreventive efficacy of morin was estimated on tissue lipid peroxidation and antioxidant status, which are used as biomarkers in 1,2- dimethylhydrazine – induced colon carcinogenesis in a rat model [7].

Protective effects of morin during the therapy of reperfusion injury of kidney in the laboratory rats were studied by [8]. It has beneficial effects of morin on CCl_4 - induced acute hepatotoxicity in rats.

*Corresponding author e-mail: mschitra21@yahoo.com

Morin, a plant – derived flavonoid, has been reported to exhibit a wide range of pharmacological properties, it has protective effect on hepatic fibrosis induced by dimethylnitrosamine (DMN) in rats [9]. It has ability to modulate RS induced NF-kappa B activation through its scavenging activity. Results indicate that morin neutralized RS in vitro and inhibited t- BHP – induced RS generation [10]. It regulates the Janus kinase-signal transducers and activators of transcription (JAKs / STATs) pathway in focal segmental glomerulosclerosis.

Materials and Methods

Experimental Animals

Healthy adult male albino Wistar rats, bred and reared in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University were used for the experiment. The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India). Animal handling and experimental procedures were approved by the Institutional Animal Ethical Committee, Annamalai University.

Chemicals

Morin was purchased from Sigma-Aldrich. All other chemicals and solvents were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai and Himedia Laboratories Pvt. Ltd., Mumbai, India.

Hepatotoxicity

Hepatotoxicity was induced by single intraperitoneal (i.p) injection of D-galactosamine (400 mg/kg body wt.), dissolved in physiological saline.

Experimental Design

The animals were randomly divided into four groups of six animals each. The PCA and silymarin were dissolved in 0.9% saline vehicle solution and fed by intubation.

Group I: Control rats received 0.9% saline only
Group II: Control + morin (50mg/kg body weight)
Group III: D-GalN control (400 mg/kg body weight)
Group IV: D-GalN + morin (50 mg/kg body weight)

After 21 days of treatment, the animals were fasted for 12 h, and sacrificed by cervical dislocation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of various biochemical parameters. Tissues were removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice-cold containers. Erythrocytes were also prepared for the estimation of various biochemical preparations.

Tissue homogenate preparation

Liver and kidney tissues (250 mg) were sliced into pieces and homogenised in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Enzymatic and non-enzymatic antioxidants

Superoxide dismutase [11], catalase [12], Glutathione peroxidise [13], reduced glutathione [14], ascorbic acid [15] and α–Tocopherol [16] were analyzed in this study. Total RNA was isolated using guanidium thiocyanate-chloroform-phenol method by reverse transcriptase-polymerase chain reaction.

Results and Discussion

The activities of the antioxidant enzymes were remarkably decreased in erythrocyte and tissues of D-GalN administered group. In response to Morin treatment, the activities of the enzymatic antioxidants significantly increased near normalcy.

Tables 1 and 2 showed the levels of non-enzymatic antioxidants and the levels of non-enzymatic antioxidants in D-GalN hepatotoxic rats significantly decreased and treatment with Morin, significantly increased the antioxidant levels.

Table 1. Effect of morin on the activities of SOD, CAT and GPx in experimental rats

Groups	SOD (U*/mg protein)	CAT ($U^{\#}$/mg protein)	GPx($U^{\$}$/mg protein)
I	8.35 ± 1.47^{a}	77.09 ± 5.75^{a}	9.16 ± 0.46^{a}
II	9.57 ± 0.85^{b}	81.92 ± 8.11^{b}	9.77 ± 0.80^{b}
III	4.12 ± 0.45^{c}	51.23 ± 3.82^{c}	5.32 ± 0.58^{c}
IV	5.85 ± 0.87^{d}	61.46 ± 4.58^{d}	6.51 ± 0.30^{d}

U* = enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.
$U^{\#}$ = μmole of H_2O_2 consumed/minute.
$U^{\$}$ = μg of GSH utilized/minute.
Values are given as means ± SD 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 vitamin C and Vitamin E in the experimental rats

Groups	Vitamin C(mg/100 g wet tissue)	Vitamin E (mg/100 g wet tissue)
I	0.70 ± 0.06^{a}	5.82 ± 0.37^{a}
II	0.68 ± 0.06^{a}	5.99 ± 0.41^{a}
III	0.28 ± 0.03^{b}	2.30 ± 0.26^{b}
IV	0.40 ± 0.08^{c}	3.76 ± 0.37^{c}

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

Effect of morin on α-SMA (Fig.1) showed mRNA expression levels of α-SMA in liver of control and experimental rats. The transcript analysis of 4 different groups revealed notable increase in the mRNA expression of α-SMA in the liver of D-galactosamine rats (Group 3) when compared to control rats (Group 1). While morin supplementation showed significant down regulation of α-SMA levels when compared with D-galactosamine rats (group 4).

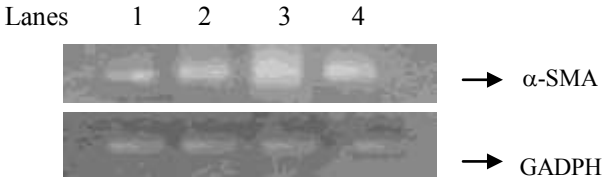

Figure 1. Effect of drug on α-SMA mRNA level in the rat liver. Photograph shows agarose gel electrophorotogram of mRNA level.

Lipid peroxidation is commonly used as an index for measuring the damage that occurs in cell membrane as a result of free radical generation. Many hepatotoxins intially injure the hepatocyte plasma membrane. Moreover, alteration of this membrane constitutes the irreversible step in the development of most forms of lethal hepatocytes damage [17]. In particular, the peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of D-GalN [18]. In the presence of molecular oxygen, such radicals attack unsaturated fatty acids in membrane and organelles to produce lipid epoxides and peroxides.

Lipid peroxidation is initiated by free radicals and is the oxidative deterioration of poly unsaturated fatty acids [19]. There was an increase in the levels of lipid peroxidation in tissue after D-GalN which is in line with the findings of Mourella and Meza [20], The increase in the levels of TBARS and lipid hydroperoxides (LOOH) indicate enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals [21]. The peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of D-GalN. Treatment with morin causes significant decrease in the levels of TBARS and LOOH in rats intoxicated with D-GalN suggesting that morin may exert a stabilizing action on liver cell membrane. In line with our findings Hung *et al* [9] also reported that morin inhibits the progress of lipid peroxidation in CCl_4 administered rats, thus demonstrating the potent antioxidant and antiperoxidative effects of morin.

The primary defense against oxidative stress in the tissue rests with antioxidants. Therefore, these antioxidants are expected to be consumed by enhanced radical reactions [6]. Significant decrease in the activity of tissue defense system after intraperitonial administration of D-GalN has already been reported [22].

SOD which converts superoxide radicals to H_2O_2 is widely distributed in cells having oxidative metabolism and is believed to protect such cells against the toxic effects of superoxide anion [23]. Superoxide anions are known to exert destructive effects on cellular components with lipid peroxidation being one such consequence. CAT is a heme protein, which catalyses the direct degradation of hydrogen peroxide to water. It protects the cellular constituents against oxidative damage. The decreased activities of these antiperoxidative enzymes during D-GalN administered in our study are compatible with other studies [24-25]. The decreased activity of these enzymatic antioxidants may be due to the accumulation of H_2O_2 which in turn causes the inhibition of these enzymes [26]. The reduced activities of these enzymes were normalized upon treatment with morin.

GPx catalyses the reduction of hydrogen peroxide and hydroperoxide to non-toxic products and scavenges the highly reactive lipid peroxides in the aqueous phase of cell membrane. GPx and the cellular NADPH-generating mechanisms together form a system for removing hydroperoxides from the cell [26]. The decreased activity of GPx in D-GalN intoxicated group might be correlated to the

decreased availability of its substrate GSH. After oral treatment with morin, the GPx levels significantly improved to near normal.

GSH, important endogenous antioxidant system is found in particularly high concentration in liver and is known to have key function in protective processes [23]. The protective role of GSH against cellular lipid peroxidation has been well documented [27]. Low concentration of GSH has been implicated in D-GalN in induced hepatitis [28]. Cellular GSH depletion is closely related to the lipid peroxidation and disturbance of Ca^{2+} induced by toxic agents [7]. An observed increase in tissue GSH content in treated group shows that the morin tends to prevent the tissue depletion of GSH.

Vit C, acts in tissues, involving reactive oxygen species in aqueous phase [29]. It has been reported that the tissue concentration of Vit C is a good indicator of oxidative stress. During liver injury the levels of Vit C was found to be significantly decreased which may be due to decreased level of glutathione[19] . Vit E, a principle lipid soluble antioxidant in cell membranes protects critical cellular structures against oxidative damage [30]. It inhibits ROS – induced generation of lipid peroxyl radicals thereby protecting cells from lipid peroxidation [31]. Supplementation of morin effectively decreased level of collagen and αSMA expression in liver D-galactosamine rats, which might be due to reduced the oxidative stress, decrease the collagen content and reduce the bundles of collagen fibers [32].

Conclusion

The present results suggested that morin have potential antioxidative effect in the D-galactosamine induced toxicity and it act as free radical scavenger to reduce lipid peroxidation and induces the enzymic and non-enzymic antioxidants.The mechanism of action of morin against hepatotoxicity is to be worked out in future research work.

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