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LETTERS

Morin augmented the metabolism and detoxification of ethanol: effects on TGF-β and the Collagen accumulation

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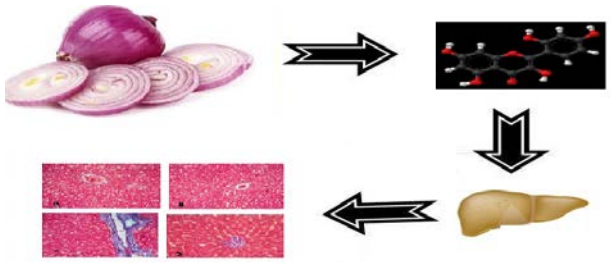
ABSTRACT



The present study was conducted to evaluate the effects of morin on the metabolism and detoxification of ethanol in the liver fibrosis. Male albino Wistar rats were divided into four groups as follows: Group 1 rats received isocaloric glucose every day, Group 2 rats received morin (60 mg/kg BW/day) everyday during the post 30 days of the experimental period; Group3received ethanol (6 g/kg BW/day) everyday for 60days, Group 4 ethanol fed rats treated with morin (60 mg/kg BW/day) for post 30 days. Ethanol treated rats showed increased levels of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA), Phospholipids (PL), activities of phase I enzymes, transforming growth factor-β1 (TGF-β1), decrease in alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and phase II enzymes. Liver fibrosis in the ethanol-fed rats as evidence by Masson's Trichrome staining. Ethanol fed rats treated with morin significant normalize the levels/activities of lipids, phase I enzymes, ADH, ALDH, phase II enzymes, TGF-β1 and collagen in liver. Thus morin curtail ethanol induced liver fibrosis and the results were supported with *In vitro* antioxidant and free radical scavenging activities of morin.

Keywords: Ethanol, liver fibrosis, morin, transforming growth factor, Masson'strichrome stain

INTRODUCTION

Liver is the main organ responsible for detoxifying and metabolizing a variety of exogenous as well as endogenous compounds. The enzymes responsible for the detoxification are primarily expressed in hepatocytes and mainly divided into two groups: Phase I and Phase II enzymes. The phase I enzymes are predominantly from the P450 family of genes, whose general function is to add polar groups, such as hydroxyl groups, to lipophilic molecules thus rendering them more hydrophilic.[1] The main function of the phase II enzymes is to covalently attach a water soluble moiety to the polar group added by the phase I enzymes such as glutathione. This usually renders the compound less reactive.[2] If the phase II reaction is impaired for some reason, or the phase I reaction is induced, this may leave the organism with an excess of reactive molecules from the phase I reactions, which can be detrimental. This can occur in the case of hepatotoxicity. When

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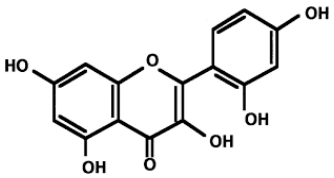
reactive metabolites of the parent compounds are formed, which subsequently negatively affect cellular functions.[3]

Alcoholic liver disease (ALD) is one of the most common causes of liver diseases worldwide increasing day by day, especially in the developing country like India and currently there is no effective medicine.[4] ALD presents initially as acute inflammation then progresses to fatty liver, alcoholic hepatitis and ultimately leads to fibrosis and cirrhosis.[5] Three major pathways for alcohol metabolism exist in the liver e.g., alcohol dehydrogenase in the cytosol, microsomal ethanol oxidizing system in the endoplasmic reticulum and aldehyde oxidase in the mitochondria. Dysregulation of above pathways lead to over production of reactive oxygen species (ROS), including superoxide, peroxide and hydroxyl radical,[6] which can causes complete degradation of lipids, proteins, and nucleic acids.[7] Besides, the major metabolic product of alcohol, acetaldehyde, damages hepatocytes and activates hepatic stellate cells trigger the production of profibrogenic cytokines, such as transforming growth factor-β1(TGF-β1), which can activates that in turn triggers inflammatory and fibrogenic signals.[8]

Herbal medicines have long been used as therapy for liver fibrosis and many are now being collected and examined in an attempt to identify possible sources of anti-liver fibrosis.[9] Natural compounds, because of their structural diversity, provide a good opportunity for screening of anti-liver fibrosis agents. Based on these reports, it would be great interest to determine the effects of morin on the ethanol induced hepatic fibrosis. Morin (2',3,4,5,7- pentahydroxyflavone) is a bioflavonoid found in the Moraceae family, mostly found in different herbs and fruits including onion, seed weeds, mill, fig, almond, red wine and Osage orange, which is used as herbal medicines, and has been suggested to act as a food preservative.[10] It has wide biological activities, including antioxidant, anti-inflammatory, chemopreventive, hepatic protection and cardioprotective.[11,12,13] Therefore, in the present study, we have evaluated the therapeutic efficacy of morin on alcohol-induced liver fibrosis and its modulatory role in detoxification enzymes.



MATERIAL AND METHODS

Ethanol and morin were purchased from Hi Media Laboratories Private Limited, Mumbai, India. TGF-β antibody was purchased from Santa-cruz Biotechnology, USA. Anti-mouse secondary antibody was obtained from Genei, Bangalore, India. All other chemicals and solvents used were of analytical grade and purchased from Sisco Research Laboratories, Mumbai, India.

Animals

Adult male albino Wistar rats (150-170 g) were procured from the Central Animal House, Rajah Muthiah Medical College and Hospital (RMMC&H), Annamalai University. The rats were housed in plastic cages under controlled conditions of 12-h light-dark cycle, 50% humidity and temperature of 28º C. All the animals were fed standard pellet diet and water *ad libitum*. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, RMMC&H, Annamalai University and animals were cared for in accordance with the Indian National Law on animal care and use.

Experimental design

The animals were divided into four groups of 6 rats each. Rats in groups 1 and 2 received isocaloric glucose from a 40% glucose solution. Animals in groups 3 and 4 received 20% ethanol (equivalent to 6 g/kg/ BW) as an aqueous solution by intragastric intubation for 60 days. In addition to isocaloric glucose and ethanol groups 2 and 4 animals received aqueous solution of morin (60 mg/kg BW) for the last 30 days of the experiment.[12] The experimental protocol is represented as below

Group 1: Control rats, received isocaloric glucose from a 40% stock glucose solution twice in a day for a period of 60 days.

Group 2: Control rats, received glucose from a 40% stock glucose solution twice daily, which was isocaloric to ethanol and morin (60 mg/kg BW) from the 30th day along with glucose in the morning.

Group 3: Rats received ethanol (6 g/kg BW) from 20% stock solution twice in a day for a period of 60 days.

Group 4: Rats received ethanol (6 g/kg BW) twice in a day as in group 3 and morin was administrated (60 mg/kg BW) from the 30th day along with ethanol in the morning.

Processing of Blood and tissue samples

Preparation of tissue homogenate

Immediately after sacrifice, the liver was quickly excised, rinsed with saline, blotted dry on filter paper and weighed. Subsequently 10% (w/v) tissue was taken with appropriate buffer to prepare tissue homogenates with homogenizer. The supernatants were used for the various biochemical estimations.

Preparation of cytoslic and microsomal fractions

Cytosolic and microsomal fractions were prepared from tissues by homogenizing with 0.25M sucrose and centrifuged at 9000×g for 20 min. The supernatant fluid was collected, 0.2 mL(v/v) of 0.1 M $CaCl_2$ in sucrose was added to each, and the samples were kept on ice for 30 min, centrifuged at 27,000×g for 20 min, which yielded clear cytosolic fractions that were promptly assayed for phase II enzymes and ADH. Microsomal pellets were washed twice by suspending in 7 mL of 10 mMTrisHCl (pH 7.4) in 0.25 M sucrose, centrifuged at 9000×g for 20 min, which yielded microsomal fractions, that were promptly assayed for phase I enzymes.

Biochemical estimations

***In vitro* antioxidant and free radical scavenging assay**

The ability of morin in scavenging the stable free radical,DPPH was determinedby*in-vitro*.[14] Total antioxidant potential of morin was determined by scavenging $ABTS^{+}$, as described by the method of Miller.[15] Superoxide anion ($O_2^{\bullet}$) and Hydroxyl radical ($OH^{\bullet}$) scavenging activity of morin was determined.[15,16]

Estimation of lipids in the liver

Lipids were extracted by the method of Folchet al.[17] (1957) using chloroform- ethanol mixture (2:1 v/v). The levels of total cholesterol (TC) were measured by the method of Siedel,*et al.*[18] and triglycerides (TG) by the method of Foster and Dunn.[19] Free fatty acids (FFA) and phospholipids (PL) were evaluated according to the methods of Falholt*et al.*[20] and Zilversmit& Davis[21] respectively.

Assay of alcohol metabolizing enzyme

Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) were assayed in the liver homogenate by the method of Agarwal and Goedde.[22]

Assay of phase I and phase II enzymes

Cytochrome P450 and cytochrome b5 were determined by the method of Omura and Sato.[23] NADPH-cytochrome b5 reductase was assayed by measuring the rate of oxidation of NADPH at 340 nm. Cytochrome P4502E1 and NADH-cytochrome P450 reductase activity were assayed by the methods of Watt et al.[24,25] and Mihara and Sato.[26] DT-diaphorase and glutathiones-transferase activities were assayed by using the methods of Ernster*et al.*[27] and Habig*et al.* respectively.[28]

Western blot analysis of TGF-β1 in the liver

Liver samples were homogenized in an 1ml ice-cold buffer (20 mMTris-HCl, pH 7.4, containing 150 mM sodium chloride, 1 mM ethylene diamine tetra-acetic acid (EDTA), 0.5% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 1mM phenyl methosulfonyl fluoride and 10 µL of protease inhibitor cocktail) and then centrifuged at 10,000 xg for 15 min at 4° C. The 50 µg of solubilized protein was electrophoresed by SDS-PAGE (12% gel) electrophoresis and then blotted onto a nitrocellulose membrane. After 1 h, the membrane was incubated in a blocking buffer (3% BSA in Tris-buffered saline, pH 7.5) containing 0.1% Tween-20 and then probed with the primary antibody (TGF-β1, 1:1000 diluted in blocking buffer) at 4 °C overnight. The membrane was washed three times with 0.1% TBST for 5 min and then incubated with HRP-conjugated anti-mouse antibody (1:1000 dilution) for 2 h at room temperature. β-actin was used as the house-keeping control (1:1000 dilution). The bands were perceived by color reaction with H_2O_2 and then scanned using image J Software.

Identification of Collagen

Liver was perfused and harvested for histological study. Liver was fixed in neutral buffered formalin (4% formaldehyde in phosphate buffered saline (PBS)). The tissue was later sectioned using a microtome, dehydrated with a graded series of ethanol and embedded in paraffin wax. Sections of thickness (5µm) were cut and stained with Masson's trichrome for the identification of collagen, later the sections were observed under light microscope.

Statistical analysis

Data were analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SPSS for Windows (17.0; SPSS Inc., Chicago, IL, USA). Results are presented as means ± SD of six rats in each group. Values of $P<0.05$ were regarded as statistically significant.

RESULTS

In vitro antioxidants and free radical scavenging activities of morin

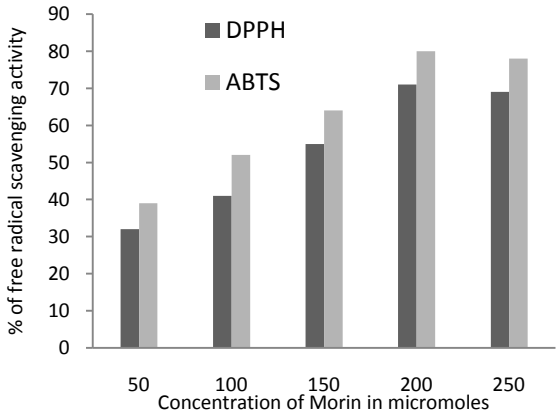


Figure 1. *Invitro* scavenging effect of morin on free radical, 1, 1-diphenyl-2-picryl-hydrazyl radical ($DPPH^{\bullet}$), 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{+}$). Values are the average of triplicate experiments

Figure 1 depicts the percentage *in vitro* scavenging effects of morin on DPPH and ABTS respectively. Morin scavenged $DPPH^{\bullet}$ and $ABTS^{\bullet}$ in a concentration-dependent manner (50, 100, 150, 200 and 250 µM). The percentage scavenging effects of morin at various concentrations (50, 100, 150,200 and 250 µM/ml) on DPPH were found to be 32, 41, 55, 71 and 69% respectively. At the concentration of 200 µM morin scavenged of 71% of DPPH. The percentage scavenging effect of morin on ABTS at various concentrations (50, 100, 150, 200 and 250 µM) were found to be 39, 52, 64, 80 and 78% respectively. The maximum percentage scavenging effect of morin on ABTS was found to be 80 at 200 µM/ml of morin.

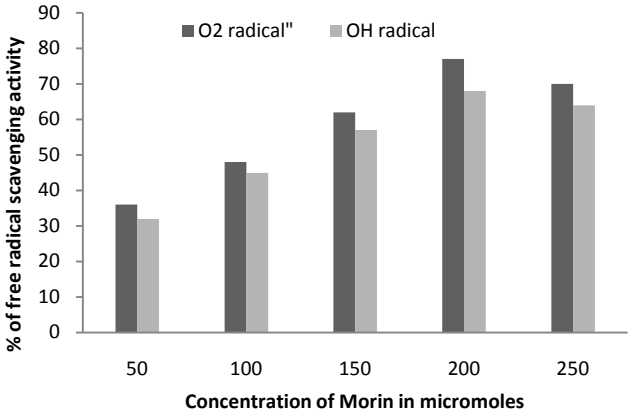


Figure 2. *In vitro* scavenging effects of morin on superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet-}$). Values are the average of triplicate experiments

Figure 2 represents the percentage in vitro scavenging effects of morin on O_2 and OH. Morin scavenged these free radicals in a concentration-dependent manner (50, 100, 150, 200 and 250µM). The percentage scavenging activity of morin increased with increasing concentration. The percentage scavenging effects of morin on O_2 at various concentrations of morin (50, 100, 150, 200 and 250µM) were found to be 36, 48, 62, 77 and 70% respectively. Furthermore, morin showed OH scavenging effect in a dose dependent manner (32, 45, 57, 68 and 64 for 50, 100, 150, 200 and 250 µM). Thus, morin at the concentration of 200 µM showed the highest percentage O_2 scavenging activity of 77% and OH scavenging activity of 68%.

Effect of morin on the levels of TC, TG, FFA and PL in the liver

The levels of TC, TG, FFA and PL were depicted in Table1.Ethanol fed rats (Group 3) showed significant elevated levels of lipids such as TC, TG, FFA and PL in the liver when compared to the control group (Group 1). The ethanol fed rats co-treated with morin (Group IV) significantly ($p<0.05$) reduced the levels of TC, TG, FFA and PL in the liver when compared to the untreated ethanol fed rats (group 3). Control rats treated with morin (Group 2) did not show any significant biochemical changes when compared to the normal control rats.

Effect of morin on alcohol metabolizing enzymes

Table 2 reveals the effect of morin and ethanol on the activities of the alcohol metabolizing enzymes in control and experimental rats. The activities of ADH and ALDH in liver were significantly decreased ($p< 0.05$) in ethanol-fed (Group 2) rats as compared to the control rats (Group 1). Whereas supplementation of morin to ethanol-fed rats (Group 4), significantly elevated the activities of ADH and ALDH when compared to the untreated ethanol fed rats (Groups 3).

Effect of morin on changes in the activities of xenobiotic metabolising enzymes

The activities of xenobiotic metabolising enzymes, phase I and phase II were given in tables 3, 4 and 5 respectively. There was significant ($p<0.05$) increased in the activities of phase I and decreased phase II xenobiotic metabolising enzymes in the liver of ethanol fed rats (Group 3) when compared to the control group (Group 1). The ethanol fed rats co-treatment with morin (Group 4) significantly ($p<0.05$) modulates the levels phase I and phase II xenobiotic metabolising enzymes in the liver when compared to the untreated ethanol fed rats (Group 3). Control rats treated with morin (Group 2) alone do not show any significant changes in the activities of detoxification enzymes.

Effect of morin on TGF-β

Fig. 3 represents the Western blot analysis of TGF-β1in the liver. There was increased expression of TGF-β1in the liver of ethanol fed rats when compared to control group. Whereas the expression of TGF-β1 was significantly lowered in the ethanol fed rats treated with morin.

Identification of collagen

Hepatic fibrosis was studied histologically using Masson's trichrome staining (Fig. 4). Control and control rats treated with morin showed normal collagen deposition (Fig. 4A &B) and the architecture of hepatic lobules are complete. Ethanol treated rat liver, fibrosis is seen with bluish circular staining pattern around the portal triad (Fig. 4C). The portal triad is surrounded by well-formed collagen bundle. The lobular architecture is distorted. Marked reduction in collagen is observed in the ethanol fed rats treated with morin (Fig. 4D).

Table 1: Effects of morin on the levels of lipids in liver of control and experimental rats

Groups	mg/g tissue			
	TC	TG	PL	FFA
Control	3.45 ± 0.29[a]	3.69 ± 0.31[a]	17.88 ± 1.49[a,c]	6.45 ± 0.58[a]
Control +Morin (60 mg/kg BW)	3.36 ± 0.17[a]	3.54 ± 0.25[a]	17.10 ± 1.12[a]	6.40 ± 0.48[a]
Ethanol (6 g/kg BW)	5.12 ± 0.51[b]	5.71 ± 0.49[b]	25.60 ± 1.89[b]	10.33 ± 1.37[b]
Ethanol + Morin (60 mg/kg BW)	3.96 ± 0.26[c]	4.16 ± 0.39[c]	19.42 ± 1.62[c]	7.60 ± 0.65[c]

Values are means ± SD for six rats.Values not sharing a common superscript letters differ significantly at $p<0.05$. ANOVA followed by DMRT.

Table 2: Effect of morin on the liver alcohol metabolizing enzymes in control and experimental rats

Groups	ADH (µmoles of NAD utilized/min/mg protein)	ALDH (µmoles of NAD utilized/min/mg protein)
Control	0.98 ± 0.09[a]	1.39 ± 0.14[a]
Control + Morin (60mg/kg BW)	0.95 ± 0.07[a]	1.26 ± 0.10[a]
Ethanol (6g/kg BW)	0.47 ± 0.05[b]	0.68 ± 0.09[b]
Ethanol + Morin (60mg/kg BW)	0.76 ± 0.06[c]	0.95 ± 0.08[c]

Values are means ± SD for six rats.Values not sharing a common superscript letters differ significantly at $p<0.05$. ANOVA followed by DMRT

Table 3. Effects of morin on the activities of liver microsomal phase I enzymes in controland experimental rats

Groups	NADPH-CytP450 reductase (µmol of NADPH oxidized/min/mg protein)	NADH-Cyt b5 reductase (µmol of ferric cyanide reduced/min/mg protein)
Control	56.73 ± 3.10[a]	15.60 ± 1.45[a]
Control + Morin (60mg/kg BW)	59.10 ± 3.65[a]	16.33 ± 1.50[a]
Ethanol (6g/kg BW)	79.03 ± 7.08[b]	33.23 ± 2.85[b]
Ethanol + Morin (60mg/kg BW)	65.23 ± 5.45[c]	20.83 ± 1.59[c]

Values are means ± SD for six rats. Values not sharing a common superscript letters differ significantly at $p<0.05$. ANOVA followed by DMRT

Table 4. Effect of morin on the liver microsomal phase I enzymes in the control and experimental rats

Groups	Cytochrome P450 (µmoles/mg protein)	Cytochrome b5 (µmoles/mg protein)	Cytochrome P4502E1 (m moles of p-nitrocatechol liberated/min/mg protein)
Control	5.82 ± 0.41[a]	2.22 ± 0.19[a]	5.82 ± 0.52[a]
Control + Morin (60mg/kg BW)	5.90 ± 0.60[a]	2.20 ± 0.10[a]	5.90 ± 0.42[a]
Ethanol (6g/kg BW)	11.28 ± 1.05[b]	4.48 ± 0.42[b]	13.19 ± 1.10[b]
Ethanol + Morin (60mg/kg BW)	7.15 ± 0.63[c]	3.30 ± 0.27[c]	7.95 ± 0.85[c]

Values are means ± SD for six rats. Values not sharing a common superscript letters differ significantly at p<0.05. ANOVA followed by DMRT.

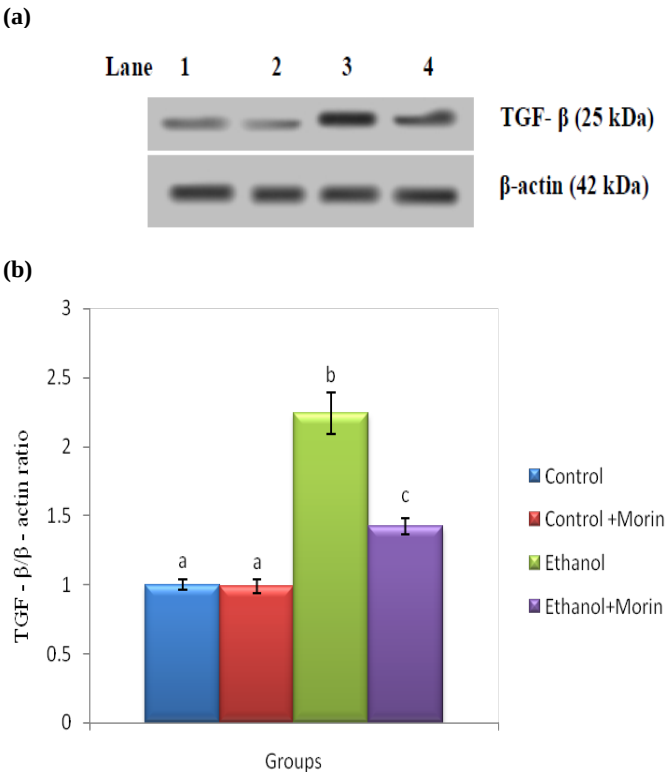


Figure 3. Effect of morin on TGF-β protein expression in the liver of control and experimental rats (a) TGF-β protein expression by western blot Lane 1: Control; Lane 2: Control + Morin (60 mg/kg BW) Lane 3: Ethanol; Lane 4: Ethanol + Morin (60 mg/kg BW) (b) Band intensities scanned by densitometer.

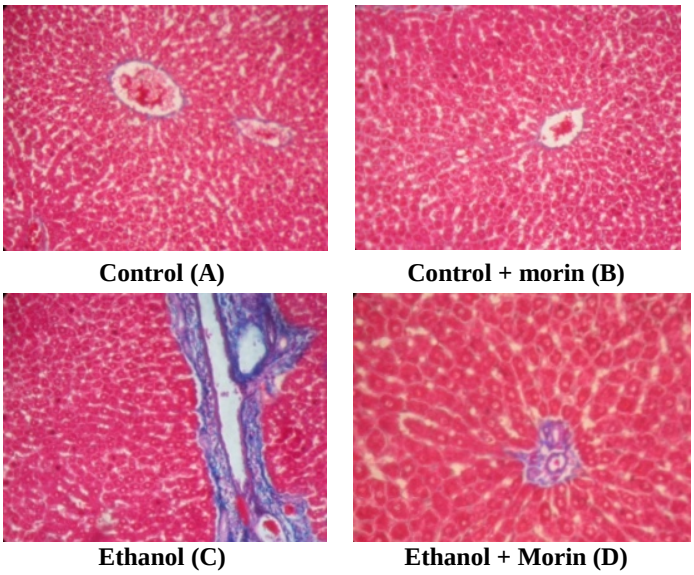


Figure 4. Histological section of liver Biopsy using Masson's trichrome staining.

DISCUSSION

Animal model for acute ethanol-induced hepatotoxicity in human drinkers have been well documented. In our current study rats were orally administered at the doses of 6 g/kg ethanol is the ideal model to the study of ethanol toxicity. Alcohol is initially oxidized into acetaldehyde then into acetate with the help of NAD^+ dependent cytosolic ADH and mitochondrial ALDH. Acetaldehyde, a highly toxic metabolite of ethanol, plays a crucial role in the pathogenesis of ALD,[29] the accumulation of acetaldehyde in the liver after chronic alcohol ingestion is determined by its formation and removal rates as catalyzed by ADH and ALDH, respectively.[30] Due to the functional diversity of hepatocytes, alterations in the levels of theses specific enzymes are used as an index of intoxication. The prolonged ethanol administration could deplete NAD^+ that could be responsible for reduced activities of ADH and ALDH. Furthermore, rise in the ethanol concentration could cause hepatic NADH accumulation. The reducing equivalents impede tricarboxylic acid cycle (TCA) activity and fatty acid oxidation, decreased mitochondrial fatty acid β-oxidation and increased endogenous fatty acid synthesis or enhanced delivery of fatty acids to the liver. Liver is a vital organ participates in the uptake, oxidation and metabolic conversion of FFA, synthesis of TC, PL. Previous reports have shown that ethanol increases lipid levels in the liver.[31]All these lead to a marked accumulation of fat in the liver during chronic alcohol consumption followed by fatty liver, hepatitis, fibrosis, and cirrhosis. In the present study, ethanol feeding resulted in decreased the activities of ADH and ALDH. Supplementation of morin to the ethanol-fed rats increased the activities of ADH and ALDH. Lowering of blood-ethanol concentration by morin might be attributed to enhanced ethanol metabolism by a rise in activities of ADH and ALDH. However, the precise mechanism remains unclear.

Lowering of tissue lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of liver disease. The reduced tissue lipids (TC, TG, PL and FFA) in morin co-treated rats might be due to increased in the mobilization and hydrolysis of certain lipoproteins for their selective uptake and metabolism by different tissues. Phenolic compounds have the ability to normalize the levels of tissue lipids during diseased conditions. It was reported that the morin possesses antioxidant effects and hypolipidemic effect.[32] Therefore, the possible mechanisms by which the morin modulates lipid levels during ethanol fed condition could be attributed due to its antioxidant property. A decrease in fat accumulation in ethanol-fed rats was also observed in response to antioxidant therapy.[33] Thus, in the present study, multiple mechanisms are probably involved in the reduction of the degree of fat accumulation observed in morin co-treated rats.

The detoxification systems are highly complex, show a great amount of individual variability, and are extremely responsive to an individual. The detoxification enzymes metabolize the xenobiotic compounds, rendering them water soluble, thereby facilitating its excrete from the body.[34] It is now known one mechanism the body uses is a battery of enzymes, each with broad specificity, to manage this challenge. CYP450, a key enzyme in biotransformation, belongs to the microsomal hemoproteins, uses oxygen as a cofactor, NADH, to add a reactive group, such as a hydroxyl radical.[35] CYP2E1 plays an important role in the catalysis of lipid peroxidation and production of reactive oxygen intermediates such as H_2O_2 in higher amounts relative to other P450 isoforms by the regulation of NADPH oxidase activity. CYP2E1 oxidizes ethanol to generate many toxic products, such as acetaldehyde, 1-hydroxyethyl radical, and other ROS, such as O_2, H_2O_2 and OH as well as the lipid peroxidation-end product MDA.[36] Our results also showed increased activities of CYP450, cytochrome b5, NADH-cytochrome b5 reductase, NADPH-cytochrome P450 reductase, cytochrome P4502E1 in the ethanol-fed rats. In this context, Jayachitra and Nalini[37] have reported an increase in the activities of cytochrome b5, NADPH cytochrome P450 reductase and NADH-cytochrome b5 reductase activities in the liver of ethanol-fed rats. It has been shown that chronic ethanol consumption increases CYP2E1 activity in the liver. Co-treatment of morin to the ethanol-fed rats decreased the activities of cytochrome P450, cytochrome b5, NADPH-cytochrome P450 reductase, NADH-cytochrome b5 reductase and cytochrome P4502E1 in the liver, which may be because of the modulatory effect of morin on cytochrome P450 dependent monooxygenases, the primary enzyme involved in the metabolism of many xenobiotics.[38,39] The action of morin on the phase I enzymes may be, at different time points, resulting in significant protection against ethanol induced toxicity. The phase II detoxification enzymes, act by metabolizing xenobiotic and endobiotic compounds rendering them water soluble, thereby facilitating their removal from the body. The GST enzymes are soluble proteins predominantly found in the cytosol of hepatocytes and catalyze the conjugation of a variety of compounds with the endogenous tripeptide, GSH. GST is subject to activation by endogenous metabolism of drugs.

Quinone reductase (QR) also called DT-diaphorase, is an enzyme present in the hepatic cytosol that produces NAD^+ from NADH. DT-diaphorase is generally induced concomitantly with other phase-II detoxifying enzymes. Morin supplementation enhanced the DT-diaphorase and GST activities in ethanol-fed rats. It has shown that inducers of DT-diaphorase can enhance the regeneration of NAD^+ and thereby enhance the *in vivo* metabolism of ethanol and decrease hepatotoxicity,[40] Hence, a decrease in the activities of phase II enzymes in ethanol fed rats would lead to accumulation of toxic substances resulting oxidative damage. Ethanol fed rats treated with morin significantly restored the phase II enzymes. The multifunctional inducers include many of the flavonoid molecules found in fruits and vegetables induce several Phase II enzymes while decreasing Phase I activity. In general, this increase in Phase II supports better detoxification in an individual and helps to promote and maintain a healthy balance between Phase I and Phase II activities. The enhancement of Phase II activity has been proposed to explain, at least in part, the ability of flavonoid molecules present in the fruits and vegetables.

Ethanol induced reactive oxygen species can up regulate the production of TGF-β. TGF-β1 is a potent stimulus for the production of collagen; increased collagen accumulation is a hallmark of fibrosis which can be determined by staining.[40] Masson's trichrome staining is used in this study to identify the collagen accumulation. This is concomitant with the increased TGF-β1expression. In order to know free radical scavenging activity of morin, we also investigated *in vitro* effects of morin on scavenging $O_2^{\bullet-}$and $OH^{\bullet-}$. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. It has been reported that $O_2^{\bullet-}$radicals directly initiate lipid peroxides.[41] $O_2^{\bullet-}$is a precursor of active free radicals that have the potential of reacting with biological macromolecules thereby inducing tissue damage. $OH^{\bullet-}$radical is chiefly responsible for lipid peroxidation, which impairs the normal function of cell membranes. Any condition which disrupts redox homeostasis produces an oxidative stress in cells where the redox steady state of the cell is altered in the direction of prooxidants that leads to the accumulation of reactive oxygen species. In the present study, it was clear that morin scavenged $O_2^{\bullet-}$ and $OH^{\bullet-}$ in a dose dependent manner. The highest percentage of scavenging effect of morin on $O_2^{\bullet-}$ and $OH^{\bullet-}$ was found to be at the concentration of 200 µM. The present findings clearly demonstrated that morin is an effective free radical scavenger against $O_2^{\bullet-}$and $OH^{\bullet-}$. Phenolic compounds from phytochemical are important low molecular mass antioxidants coming from the diet.[42] Many plant phenolics exhibited antiradical or antioxidative activity *in vitro* and *in vivo*.[43,] The intensity of antiradical activity of phenols depends on many factors such as number of hydroxyl groups bound to the aromatic ringand the number and places of double bonds in the molecule. The chemical structure of morin and other bioflavonoids can be distinguished by the presence of two aromatic rings connected by c-pyrone ring where polar hydroxyl

groups are bound in various positions. These hydroxyl groups are suggested to be responsible for the free radical scavenging properties shared by morin. The present report corroborate to our previous study, the protective effect of morin on lipid peroxidation and antioxidant in ethanol induced liver injury. Furthermore treatment of morin (60 mg/kg BW) can ameliorates the fibrogenic activity via enhancement of ethanol metabolism, modulate the xenobiotic metabolizing enzymes and down regulation of TGF-β expression. Furthermore, the antioxidant nature of morin merits its antifibrogenic effect. In conclusion, morin could be a therapeutic agent for the treatment of ALD.

CONCLUSION

In conclusion ethanol-induced liver fibrosis can be alleviated by the potential beneficial effects of morin as evidenced by the improvement with morin. Morin significantly normalize the levels/activities of lipids, phase I enzymes, ADH, ALDH, phase II enzymes, TGF-β1 and collagen in liver. Thus morin curtail ethanol induced liver fibrosis and the results were supported with *In vitro* antioxidant and free radical scavenging activities of morin.

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