ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2013; 4(1): 208-217

ORIGINAL RESEARCH ARTICLE

Beneficial Effect of Morin on Lipid Peroxidation and Antioxidant Status in Rats with Ethanol Induced Dyslipidemia and Liver Injury

Singaravelu Anbu[1] and Nadanam Saravanan*[2]

[1]Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

[2]Division of Biochemistry, Rani Meyyammai College of Nursing, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

Received 08 Nov 2012; Revised 10 Feb 2013; Accepted 22 Feb 2013

ABSTRACT

Morin, a naturally occurring bioflavonoid protects the development and progression of alcoholic liver disease (ALD). The current study aims to explore the effect of morin on ethanol-induced dyslipidemia and oxidative stress in plasma, erythrocytes and liver mitochondria of rats. Hepatotoxicity was induced in rats by administering ethanol (6g/kg BW) daily for a period of 60 days. Morin (15, 30, 60 and 120 mg/kg BW) was administered to the ethanol-fed rats after 30 days of the experimental period and the treatment was continued upto 60th day. Ethanol administered rats showed a significant elevation in plasma alanine transaminases, aspartate aminotransferases, alkaline phosphatase and γ-glutamyl transferase. Ethanol induction in rats showed significant elevated levels of lipids and altered lipid profile levels in the plasma. In addition, the levels of thiobarbituric acid-reactive substances and lipidhydroperoxides were also significantly elevated in the plasma, erythrocyte, and hepatic mitochondria of ethanol-fed rats as compared to control rats. Decreased activities/levels of superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione were observed in ethanol-administered rats. Oral administration of morin (15, 30, 60 and 120 mg/kg BW) to the ethanol-fed rats showed significant protective effects on all the biochemical parameters studied which was evidenced by histopathological studies. Oral administration of morin (60 mg/kg BW) showed its high potentiality in attenuating dyslipidemia and oxidative stress in plasma, erythrocytes and liver mitochondrial in ALD by virtue of its potent hepatoprotective, hypolidemic and antioxidant effects.

Keywords: Morin, Ethanol, dyslipidemia, Oxidative stress, erythrocytes, liver mitochondria.

1. INTRODUCTION

Liver is the primary metabolic organ responsible for the xenobiotics and it is the most common targeted organ for drugs, toxins and chemicals.[1] Ethanol is one of the most abused drugs world-wide and its chronic consumption causes accumulation of fats in the liver which leads to hepatocellular injury and damage. According to the World Health Organization reports in 2005, approximately 2 billion people were consuming alcohol worldwide and about 76 million of them were prone to alcoholic liver disease (ALD), which is responsible for the high range of mortality worldwide.[2]

Overproduction of ROS is a unifying mechanism for the liver injury. In liver, metabolism of ethanol through mitochondria and induction cytochrome P_{450} isoenzyme (CYP2E1) generates reactive oxygen species (ROS) such as hydroxyls, superoxide anions, 1-hydroxy ethyl radicals promoting oxidative stress and making imbalance in normal cellular redox status. Moreover, generated ROS reacts with lipid components of biomembrane leads to lipid peroxidation. Polyunsaturated fatty acids (PUFA) are the most susceptible target of free radical attack, which causes more damages to lipids resulting in loss of structural and functional integrity at the cellular level and eventually cell death.[3] The outcome of chronic ethanol consumption results in increased hepatic triglycerides (TG), which leads to fatty

***Corresponding Author:** Nadanam Saravanan, **Email:** saravanan_74@rediffmail.com

liver, steatosis and hepatomegaly.[4] Moreover, accumulation of fats in the liver provides them as a substrate for peroxidation reactions. Increased hypertriglycemia and altered mitochondrial redox are the key factors mediating the ALD. In circumstances of overproduction of free radicals, the body's antioxidant defense system is depleted, so that not able to inhibit the further damages caused by oxidants. Therefore, consuming foods containing antioxidants may restore this imbalance and revive the anatomy and physiology of the injured organ almost back to its normal state.[5] Dietary factors containing antioxidants are of much attention in the prevention of ALD. Thus, the identification of antioxidants, which can retard the process of lipid peroxidation by blocking the generation of free radicals, has gained much attention in recent years. Among such class of compounds, flavonoids are of much interest because of their role in the prevention of many human disease due to their wide range of pharmacological properties including antioxidant, cytoprotective, antimutagenic, anti-inflammatory and chemopreventive properties.[6] Morin (Fig 1- 3, 5, 7, 20, 40 -pentahydroxyflavone), a yellow pigment bfla v onoid constituent of fruits, vegetables and nuts are thought to be components of herbal-containing dietary supplements and used as food preservative.[7] Morin modulates the activities of the metabolic enzymes that protect various human cells[8] and cell organelles against oxidative damage.[9,10] Beside these, it has the ability to act as an agent with beneficial properties such as antioxidant, anti-inflammation, antihypertensive, hypolipidemic, anticarcinogen and chelating agent.[11,12] Despite current advances in medical management, no definite therapy for acute liver failure exists, and this abnormality remains a major public health concern. Therefore, prevention of the ALD by supporting the body against such disorders should strongly be taken into consideration. Hence, the present study aims to investigate the protective role of morin on ethanol-induced liver damage, dyslipidemia and the oxidative stress in the plasma, erythrocytes and liver mitochondria.

2. MATERIALS AND METHODS

Chemicals

Morin hydrate was purchased from Sigma Chemicals Co., St. Louis, Mo, USA. Absolute ethanol was obtained from Nellikuppam, Cuddalore District, Tamilnadu, India and all the other chemicals and solvents used were of analytical grade.

Experimental Animals and diet

Male albino Wistar rats 150-180 g were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Chidambaram, The animals were housed in polypropylene cages in a well-ventilated room and maintained in a 12-h light/12-h dark cycle, 50% humidity and 30̊ C. The animals had free access to standard pellet diet (Pranav Agro Industries Ltd, Bangalore, India) and water *ad libitum.* This study was approved by Institutional Animal Ethics Committee, Rajah Muthiah Medical College (Registration Number: 166/1999/CPCSEA,) and the study was conducted in accordance with the Guidelines of "Committee for the purpose of control and supervision on experimental animals" (CPCSEA, 2004).

Oral administration of Morin Preparation

Morin was freshly suspended in water[13]and administered to rats orally using an intragastric tube.

Experimental Design

The 56 male albino Wistar rats were divided into 7 groups; two animals from each group were used for the histological study.

Group 1 : Control rats, received isocaloric glucose from a 40% stock glucose solution twice in a day daily 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 (120mg/kg BW) from the 30th day along with glucose in the morning.

Group 3 : Rats received ethanol (6g/kg BW) from 30% stock solution twice in a day daily for a period of 60 days.

Group 4 : Rats were administered with ethanol (6kg/kg BW) twice in a day and morin (15mg/kg BW) from the 30th day along with ethanol in the morning.

Group 5-7 : Rats were treated as for group 4 but with morin equal to 30, 60 and 120 mg/ kg BW respectively.

After the experimental period of 60 days, the animals were fasted over night, anesthetized with ketamine hydrochloride (30mg/kg, i.p) and then sacrificed by cervical decapitation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 min. The blood, collected in a heparinised centrifuge tube was centrifuged at 2000 rpm for 10 min and the plasma was

separated by aspiration. Liver was excised, washed in ice-cold saline, weighed and the tissue homogenate was prepared in appropriate buffer.

Erythrocytes preparation

After the separation of plasma, the buffy coat, enriched in white cells, was removed and the remaining erythrocytes were washed three times with physiological saline and made up to a known volume. A specific volume of erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2500 rpm for 10 min and the supernatant was used for the estimation of enzymic antioxidants.

Preparation of mitochondrial fraction

Mitochondria were isolated by the method of Johnson & Lardy[14]. A 10% liver homogenate was prepared in 10mM Tris-HCl, pH 7.4 containing 0.25M sucrose. The homogenate was centrifuged at 600xg for 10min at below 4°C and the supernatant were centrifuged at 10, 000xg for 20min at 4°C. The pellets were washed with ice-cold 0.15M KCl and suspended in the buffer solution. The protein content was measured by the method of Lowry *et al*.[15]

Biochemical Estimations

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using the method of Reitman & Frankel.[16] Alkaline phosphatase(ALP) and γ-glutamyl transferase (GGT) were estimated using the method of Kind & King,[17]and Rosalki & Rau[18] respectively. Bilirubin was estimated by the method of Malloy and Evelyn.[19] Lipids were extracted from the plasma by the method of Folch et al.[20] Total cholesterol (TC) in the plasma was estimated by the enzymic method described by Zlatkis et al.[21] TG and free fatty acids (FFA) in the plasma were estimated according to the method of Foster & Dunn[22] and Falholt et al[23] respectively. Phospholipids (PL) were estimated by the method of Zilversmit & Davis[24] High density lipoprotein (HDL) was estimated by the method of Izzo et al.[25] Low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels were calculated by the method of Friedwald *et al*.[26] Purity of mitochondria was checked by measuring the activity of succinate dehydrogenase (SDH) by Slater & Bonner.[27] Lipid peroxidation by products such as thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LOOH) were measured in plasma, erythrocytes and liver mitochondria by the methods of Niehaus & Samuelsson[28] and Jiang et al[29]respectively. The activities/levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were measured by the methods described in detail elsewhere.[30]

Histopathological investigation

Liver tissue was excised, washed and placed in 10% formalin. They were later sectioned with a microtome, dehydrated in ethanol, embedded in paraffin wax. Five micrometer thick sections were stained with hematoxylin and eosin (H&E) and studied by a routine light microscope.

Statistical analysis

Results are expressed as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistical software package (SPSS for Windows, V. 13.0, Chicago, IL, USA). p-values < 0.05 were regarded as statistically significant.

3. RESULTS AND DISCUSSION

Effect of morin on hepatic marker enzymes

Effect of morin on the activities of plasma hepatic marker enzymes such as AST, ALT, ALP and GGT in the control and experimental rats were shown in (**Table 1**).The hepatic marker enzymes were significantly ($p<0.05$) elevated in ethanol-fed rats when compared to control rats. Oral administration of morin at various doses (except 15 mg/kg BW) to ethanol-fed rats significantly ($p<0.05$) decreased the activities of these hepatic marker enzymes. The level of plasma bilurubin was also increased in ethanol-fed rats, which was significantly resorted after oral treatment with morin.

Effect of morin on Lipid profile levels

(**Table 2 & 3**) showed the levels of plasma lipid profile. Ethanol-fed rats showed elevated levels of plasma TC, TG, PL, FFA, LDL and VLDL and decreased level of HDL compared to control rats. Ethanol-fed rats when treated with morin (except 15 mg/kg BW) showed significant decreased levels of plasma TC, TG, PL, FFA, LDL and VLDL and subsequently significant increased HDL levels towards normal when compared to untreated ethanol-fed rats (Group 3).

Effect of morin on oxidative stress

Effect of morin on the concentration of TBARS and LOOH in the plama, erythrocytes and liver mitochondria of control and experimental animals were given in (**Table 4**). The concentration of TBARS and LOOH in the ethanol-fed rats (Group 3) was significantly higher when compared to the control rats. Morin treatment (except 15 mg/kg BW) along with ethanol showed a protective

effect evidenced by significant ($p < 0.05$) lowered levels of TBARS and LOOH when compared to untreated ethanol-fed rats. Treatment with morin (120 mg/kg BW) to control rats (Group 2) did not alter the concentration of TBARS and LOOH.

Effect of morin on antioxidants

The activities/levels of antioxidants in the plasma, erythrocytes and liver mitochondria of ethanol-fed rats were given in (**Table 5 & 6**). Ethanol-fed rats showed decrease in the activities/levels of antioxidants. Co-treatment of morin (except 15 mg/kg BW) to ethanol-fed rats significantly improves the antioxidants when compared to the untreated ethanol-fed rats (Group 3).

Effect of morin on liver histology

Fig 2 (a-g) represents the histological sections of liver from experimental animals. Fig 2(a) shows the liver section of group 1 in which the portal triad and hepatocytes appear normal. Fig 2(b) shows the liver section of group 2 in which hepatocytes appear normal, which are arranged in trabecular pattern. Fig 2 (c) represents the liver histology of group 3 in which the portal triad is eroded and is infiltrated with inflammatory cells. Fig 2 (d) represents the liver section of group 4 hepatocytes showing microvesicular fatty change with kuppfer cell hyperplasia. Fig 2 (e) shows damaged portal triad and surrounded by inflammations (Group 5). Fig 2(f) (Group 6) and Fig 2(g) (Group 7) hepatocytes shows normal hepatocytes and are arranged in trabacualr pattern.

Table 1: Effect of morin on hepatic markers and bilirubin in control and experimental animals

Groups	Hepatic Function Markers (IU/L)				
	AST	ALT	ALP	GGT	Bilirubin (mg/dL)
Control	75.45 ± 7.08[a]	29.51 ± 2.48[a]	81.95 ± 3.63[a]	1.84 ± 0.67[a]	0.57 ± 0.09[a]
Control +Morin (120 mg/ kg BW)	72.10 ± 6.74[a]	29.64 ± 1.84[a]	82.89 ± 8.11[a]	2.06 ± 0.22[a]	0.58 ± 0.08[a]
EtOH (6g/kg BW)	169.73 ± 9.70[b]	77.27 ± 7.73[b]	159.73 ± 12.78[b]	5.02 ± 0.29[b]	1.41 ± 0.14[b]
EtOH + Morin (15 mg / kg BW)	160.44 ± 14.67[b]	72.59 ± 7.13[b]	151.04 ± 14.62[b]	4.85 ± 0.37[b]	1.33 ± 0.11[b]
EtOH + Morin (30 mg / kg BW)	106.61 ± 10.00[c]	55.56 ± 3.30[c]	124.80 ± 9.66[c]	3.93 ± 0.35[c]	0.95 ± 0.09[c]
EtOH + Morin (60 mg / kg BW/)	81.31 ± 6.88[d,a]	36.23 ± 3.55[d]	89.19 ± 7.13[d,a]	2.55 ± 0.24[d]	0.77 ± 0.04[d]
EtOH + Morin (120 mg / kg BW/)	90.06 ± 9.61[d]	41.19 ± 4.60[d]	99.25 ± 9.63[d]	2.89 ± 0.30[d]	0.80 ± 0.06[d]

Data are mean ± SD values for 6 rats in each group. Values not sharing a common superscript letter within each column differ significantly at p < 0 .05 (DMRT).

Table 2: Effect of morin on plasma lipids level in control and experimental rats

Groups	TC (mg/dL)	TG (mg/dL)	FFA (mg/dL)	PL (mg/dL)
Control	76.52 ± 7.38[a]	57.35 ± 3.99[a]	80.81 ± 5.20[a]	95.52 ± 6.60[a]
Control +Morin (120 mg/ kg BW)	75.15 ± 4.03[a]	58.00 ± 3.57[a]	82.50 ± 3.65[a]	94.50 ± 8.47[a]
EtOH (6g/kg BW)	147.99 ± 11.41[b]	145.53 ± 11.00[b]	148.02 ± 11.12[b]	156.17 ± 12.97[b]
EtOH + Morin (15 mg / kg BW)	143.63 ± 13.41[b]	141.00 ± 10.22[b]	142.67 ± 13.56[b]	150.17 ± 11.25[b]
EtOH + Morin (30 mg / kg BW)	113.96 ± 5.96[c]	97.13 ± 9.39[c]	121.16 ± 9.37[c]	129.52 ± 8.54[c]
EtOH + Morin (60 mg / kg BW/)	89.12 ± 6.94[d]	71.02 ± 5.09[d]	95.19 ± 4.42[d]	105.75 ± 8.97[d]
EtOH + Morin (120 mg / kg BW)	90.33 ± 5.97[d]	73.85 ± 6.37[d]	95.86 ± 4.59[d]	115.66 ± 9.76[d]

Data are mean ± SD values for 6 rats in each group. Values not sharing a common superscript letter within each column differ significantly at P<0 .05 (DMRT).

Table 3: Effect of morin on plasma lipid profile levels in control and experimental rats

Groups	HDL (mg /dL)	VLDL (mg/dL)	LDL (mg/dL)
Control	47.47 ± 4.45[a]	11.46 ± 0.45[a]	17.59 ± 1.53[a]
Control +Morin (120 mg/ kg BW)	46.42 ± 4.22[a]	11.60 ± 0.40[a]	17.12 ± 0.67[a]
EtOH (6g/kg BW)	19.94 ± 1.61[b]	29.10 ± 2.39[b]	98.93 ± 8.46[b]
EtOH + Morin (15 mg / kg BW)	20.21 ± 1.99[b]	28.20 ± 1.86[b]	95.22 ± 4.76[b]
EtOH + Morin (30 mg / kg BW)	29.73 ± 3.12[c]	19.42 ± 1.53[c]	64.82 ± 4.70[c]
EtOH + Morin (60 mg / kg BW/)	36.11 ± 3.33[d]	14.20 ± 1.20[d]	38.81 ± 1.94[d]
EtOH + Morin (120 mg / kg BW)	34.00 ± 2.10[d]	14.77 ± 1.8[d]	41.56 ± 2.04[d]

Data are mean ± SD values for 6 rats in each group. Values not sharing a common superscript letter within each column differ significantly at P<0 .05 (DMRT).

Table 4: Effect of morin on plasma, erythrocytes and liver mitochondria lipid peroxidative markers of control and experimental animals

Groups	Plasma		Erythrocytes		Liver mitochondria	
	TBARS (n moles/mg protein)	LOOH ($x10^{-5}$ m moles/mg Hb)	TBARS (n moles/mg Hg)	LOOH (m moles/mg Hb)	TBARS (n moles/mg protein)	LOOH (n moles/mg protein)
Control	1.51 ± 0.07^{a}	8.87 ± 0.74^{a}	1.47 ± 0.10^{a}	6.70 ± 0.57^{a}	1.01 ± 0.07^{a}	1.62 ± 0.11^{a}
Control +Morin (120 mg/ kg BW)	1.42 ± 0.05^{a}	8.66 ± 0.55^{a}	1.39 ± 0.12^{a}	6.66 ± 0.40^{a}	1.06 ± 0.10^{a}	1.61 ± 0.13^{a}
EtOH (6g/kg BW)	3.43 ± 0.29^{b}	14.85 ± 1.36^{b}	3.24 ± 0.25^{b}	12.83 ± 1.29^{b}	3.94 ± 0.12^{b}	3.86 ± 0.24^{b}
EtOH + Morin (15 mg / kg BW)	3.20 ± 0.26^{b}	13.98 ± 1.08^{b}	3.08 ± 0.31^{b}	12.78 ± 1.10^{b}	3.84 ± 0.37^{b}	3.78 ± 0.32^{b}
EtOH + Morin (30 mg / kg BW)	2.76 ± 0.28^{c}	11.72 ± 0.95^{c}	2.80 ± 0.23^{c}	10.66 ± 0.95^{c}	2.73 ± 0.33^{c}	2.90 ± 0.16^{c}
EtOH + Morin (60 mg / kg BW)	1.92 ± 0.09^{d}	$9.91 \pm 0.91^{d,a}$	1.95 ± 0.09^{d}	8.11 ± 0.76^{d}	1.40 ± 0.11^{d}	1.15 ± 0.10^{d}
EtOH + Morin (120 mg / kg BW)	2.00 ± 0.19^{d}	10.34 ± 0.92^{d}	2.06 ± 0.16^{d}	8.30 ± 0.85^{d}	1.44 ± 0.14^{d}	1.22 ± 0.12^{d}

Data are mean ± SD values for 6 rats in each group. Values not sharing a common superscript letter within each column differ significantly at P<0 .05 (DMRT).

Table 5: Effect of morin on antioxidants in plasma and erythrocytes of control and experimental rats

Groups	Plasma (U/mg of protein)				Erythrocytes (U/mg Hb)			
	SOD	Catalase	GPx	GSH (U^A)	SOD	Catalase	GPx	GSH (U^B)
Control	2.63 ± 0.04^{a}	3.70 ± 0.36^{a}	21.30 ± 1.60^{a}	29.30 ± 1.80^{a}	6.37 ± 0.60^{a}	175.78 ± 7.35^{a}	12.98 ± 1.12^{a}	3.30 ± 0.21^{a}
Control +Morin (120 mg/ kg BW)	2.75 ± 0.05^{a}	4.29 ± 0.39^{e}	20.25 ± 1.96^{a}	28.55 ± 1.64^{a}	6.29 ± 0.39^{a}	173.15 ± 5.17^{a}	12.65 ± 0.56^{a}	3.45 ± 0.15^{a}
EtOH (6g/kg BW)	1.41 ± 0.12^{b}	1.65 ± 0.15^{b}	12.25 ± 1.26^{b}	16.25 ± 1.20^{b}	3.16 ± 0.25^{b}	102.60 ± 5.92^{b}	5.13 ± 0.68^{b}	1.33 ± 0.11^{b}
EtOH + Morin (15 mg / kg BW)	1.56 ± 0.09^{c}	1.55 ± 0.12^{b}	13.15 ± 1.30^{b}	18.95 ± 1.30^{c}	3.25 ± 0.39^{b}	110.34 ± 5.92^{b}	5.36 ± 0.56^{b}	1.41 ± 0.06^{b}
EtOH + Morin (30 mg / kg BW)	1.65 ± 0.08^{c}	2.25 ± 0.22^{c}	16.55 ± 1.45^{c}	21.55 ± 2.13^{e}	4.15 ± 0.45^{c}	132.31 ± 11.66^{c}	7.89 ± 0.70^{c}	2.33 ± 0.20^{c}
EtOH + Morin (60 mg / kg BW/)	2.35 ± 0.14^{d}	3.30 ± 0.31^{d}	19.25 ± 1.43^{a}	25.90 ± 2.44^{d}	5.35 ± 0.33^{d}	160.84 ± 10.98^{d}	9.47 ± 0.77^{d}	2.85 ± 0.09^{d}
EtOH + Morin (120 mg / kg BW)	2.11 ± 0.19^{e}	3.00 ± 0.29^{d}	$18.10 \pm 1.80^{c,d}$	24.10 ± 2.15^{d}	5.10 ± 0.48^{d}	150.96 ± 13.84^{d}	9.39 ± 0.93^{d}	2.78 ± 0.31^{d}

Data are mean ± SD values for 6 rats in each group. U=Units for SOD, CAT, and GPx are amount of enzyme required for 50% inhibition of NBT reduction/minute, µmol of hydrogen peroxide utilized/minute, and µmol of glutathione utilized/minute, respectively.
U^A = mmoles/mg protein; U^B = mmoles/mg Hb
Values not sharing a common superscript letter within each column differ significantly at P<0 .05 (DMRT).

Table 6: Effect of morin on the activities/levels of antioxidants in liver mitochondrial fraction of control and experimental animals

Groups	SOD (U^A/mg protein)	GPx (U^B/mg protein)	GSH (µg/mg protein)
Control	2.65 ± 0.24^{a}	6.73 ± 0.64^{a}	14.69 ± 1.36^{a}
Control +Morin (120 mg/ kg BW)	2.69 ± 0.20^{a}	6.84 ± 0.61^{a}	14.90 ± 1.22^{a}
EtOH (6g/kg BW)	1.17 ± 0.08^{b}	4.10 ± 0.37^{b}	6.32 ± 0.62^{b}
EtOH + Morin (15 mg / kg BW)	1.20 ± 0.11^{b}	4.34 ± 0.42^{b}	6.40 ± 0.59^{b}
EtOH + Morin (30 mg / kg BW)	1.51 ± 0.13^{c}	5.14 ± 0.50^{c}	7.94 ± 0.66^{c}
EtOH + Morin (60 mg / kg BW/)	1.96 ± 0.16^{d}	5.85 ± 0.49^{d}	9.56 ± 0.64^{d}
EtOH + Morin (120 mg / kg BW)	1.82 ± 0.19^{d}	5.76 ± 0.55^{d}	8.90 ± 0.77^{d}

Data are mean ± SD values for 6 rats in each group. Values not sharing a common superscript letter within each column differ significantly at P<0 .05 (DMRT).U^A, enzyme concentration required for 50% inhibition of NBT reduction/min; U^B, µmol of reduced glutathione consumed/min

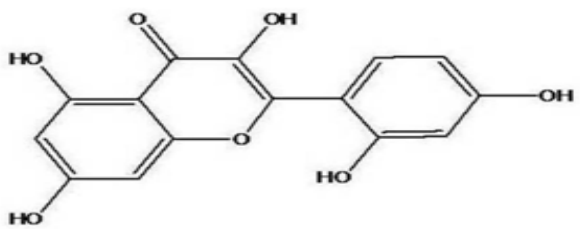

Fig 1: Chemical structure of morin (3,5,7 –trihydroxy-2-(2,4-dihydroxyphenyl)-4H-chromen-4-one

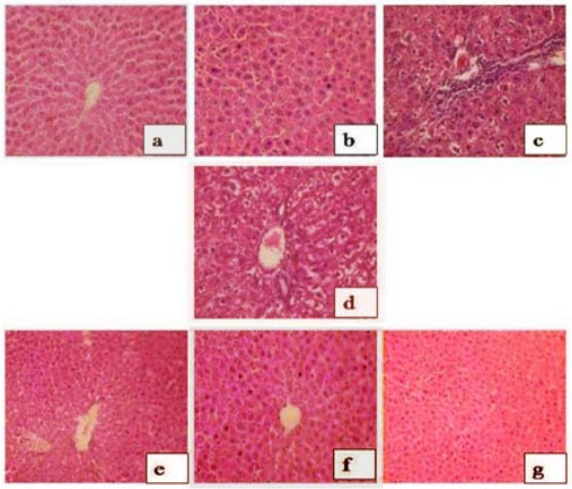

Fig 2: Histopathalogical changes of Liver

Histopathological changes of Liver (Hematoxylin-Eosin (H&E) x20. Scale bar = 100µm). (a) control rat liver, (b) control rat liver treated with morin (120 mg/kg BW), (c) ethanol fed rat liver , (d) liver of ethanol fed rats treated with morin (15 mg/kg BW), (e) liver of ethanol fed rats treated with morin (30 mg /kg BW), (f) liver of ethanol fed rat treated with morin (60 mg/ kg BW) and (g) liver of ethanol fed rats treated with morin (120mg/kg BW).

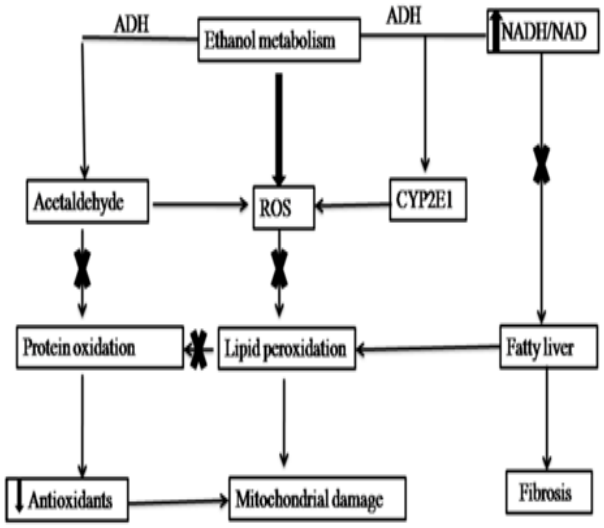

Fig 3: Mechanism of Hepatoprotective action

The toxic effects of ethanol and possible mechanism of hepatoprotective action (X) of morin
ADH- alcohol dehydrogenase; CYP2E1- cytochrome P450 isoenzyme

4. DISCUSSIONS

Liver is the most adversely affected organ after an excessive consumption of alcohol, where 90 % of alcohol metabolized. Thus, liver diseases remain one of the serious health problems due to alcohol abuse. The present investigation revealed that acute administration of ethanol resulted in a clear hepatotoxicity as evidenced by an increase in plasma ALT, AST, ALP and GGT used as indexes of liver injury, and tissue damages revealed by histological observations. The high level of hepatic markers enzymes in the plasma compartment is a central indication of the degree of damage to the liver caused by ethanol administration.[30] A prolonged destruction of hepatocytes causes an elevation of bilirubin in the serum.[31] Our data showed that co-treatment of morin (60 mg and 120 mg/kg BW/day) along with ethanol significantly reduced the activities of these enzymes, protects membrane integrity and aids in liver cells regeneration, thereby decreasing enzyme leakage. It is corroborated with the previous report, morin protects hepatocytes[8] against CCl4 induced oxidative damage.[32]

Several studies demonstrated that pro-inflammatory cytokines could partially be responsible for hypercholesterolemia and hypertriglyceridemia in ethanol-fed rats. Hyperlipidemia is vital complication of ethanol induced liver damage. Most of drugs acting on dyslipidemia are the 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG CoA reductase) Inhibitors. HMG CoA reductase catalysis the conversion of HMG CoA to mevalonate and the inhibition of this conversion is the rate limiting step in cholesterol biosynthesis. Rajasree et al.[33] have also observed the increased activity of HMG CoA reductase in the liver of ethanol-fed rats. In the present study, the increased plasma TC levels in ethanol-fed rats, which is reduced on the supplementation of morin along with ethanol indicating morin could regulate/inhibit HMG-CoA reductase activity. Recently, it has been reported that morin modulates HMG CoA reductase. [34] Elevation in TGs levels in the ethanol fed rats could be due to the increased availability of FFA and α-glycerophosphate which favors to decrease the activity of lipoprotein lipase and causes to decreased removal of TGs from circulation.[35] Furthermore, AMP-activated protein kinase (AMPK) plays a central role in the regulation of lipid metabolism. The decreased AMPK activity leads to an increase in the rate of synthesis of TG and formation of VLDL.[36]

Intake of ethanol adversely affects the plasma lipoproteins. HDL concentration decreases and LDL concentration increases in parallel with the degree of impairment of liver functions.[37] HDL is believed to play a crucial role in regulating lipid metabolism by promoting reverse cholesterol

transport and has a negative effect on the development of fatty liver. HDL helps in scavenging cholesterol from the extrahepatic tissues in the presence of lecithin cholesterol acyl transferase and brings it to the liver. Hence, the proportion of HDL is an important physiological index reflecting the potential to normalize hyperlipidemia.[38] In the present study it was found that the elevated levels of TGs, VLDL, LDL and decreased level of HDL were observed in ethanol-fed rats which were significantly modulated in morin administration. This might be due enhanced peripheral utilization and decreased synthesis of cholesterol. PLs, the vital components of bio-membranes are primary targets of peroxidation and can be altered by ethanol.[39] In the present study increased PLs levels were observed in the circulation of ethanol-fed rats, which might be due to the modification of composition, structure and stability of cell membranes. After treatment with morin there is a significant reduction in PLs which may be due to its anti-lipid peroxidative and membrane stabilizing properties.

Erythrocytes are much more vulnerable to oxidative damage because of their continuous exposure to high oxygen flux and their high concentration of PUFA.[40] The most common alteration of erythrocytes viability is membrane peroxidation and destabilizes the membrane, thereby compromising cell survivals. Acetaldehyde, produced due to the ethanol oxidation, bound to red blood cells can be distributed to various tissues and exert widespread toxic effects.[41] Increased lipid peroxidation and decreased antioxidant levels were observed in the erythrocytes may be due to the increased free radicals and intermediates of ethanol oxidation. Upon co-administration of morin to the ethanol fed rats showed decreased lipid peroxidation and increased antioxidant level which might be due to its anti lipid peroxidative and antioxidant properties.

Ethanol metabolism and mitochondrial oxidative stress play a crucial role in the pathogenesis and progression of ALD. Metabolism of ethanol and induction of CYP2E1 leads to the formation of oxidative stress through the generation of reactive oxygen species, which can start lipid peroxidation by exhausting antioxidants in the cells thereby changing the redox balance, which in turn favors increased peroxidation.[42] ROS formation within the mitochondria can alter the membrane permeability transition, suppress mitochondrial functions, such as respiration, oxidative phosphorylation, affect the inner membrane barrier functions[43]and causes release of oxidants thus hepatocytes become more vulnerable by direct interaction with the protein and lipid moieties in the membrane.

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformability, reduced lipid fluidity.[44] In the present study, the elevated levels of lipid peroxidation end products such as TBARS and LOOH in the plasma, erythrocytes and liver mitochondria of ethanol-fed rats was observed. These results are in agreement with the observations of previous researchers[44]. However, morin administered ethanol-fed rats showed significant decrease in lipid peroxidation process evidenced by decreased levels of TBARS and LOOH in plasma, erythrocytes and liver mitochondria.

In order to maintain redox homeostasis, cells have antioxidants and help to scavenge superoxide and hydroxyl radicals, thus maintain homeostasis and cell viability in response to oxidants' damage. SOD catalyses the conversion of superoxide anion to hydrogen peroxide (H_2O_2). CAT and GPx scavenge excess H_2O_2 as well as other free radicals in response to oxidative stress. The equilibrium between these antioxidants is important for the effective removal of oxidative stress in intracellular organelles. GSH is a major defense mechanism against oxidative stress within mitochondria. Depletion of mitochondrial GSH seems to be a major mechanism in inducing an imbalance of redox status within the mitochondria. Ethanol oxidized into acetaldehyde which can bind to cytosolic GSH and causes depletion of GSH entering in to mitochondria, which results in depletion of other antioxidants and enhances the susceptibility of cells to further damage. The lowered activities/levels of SOD, CAT, GPx and GSH were observed in ethanol fed rats. These antioxidants might be utilized by the cells to neutralize the produced free radicals. Treatment with morin enhanced the antioxidants in ethanol fed rats which may be due to the antilipidperoxidative and antioxidant properties of morin. The proposed mechanism of action of morin against ethanol is depicted in (Fig 3*)*.

Extensive literature survey has shown that flavonoids such as quercetin, chrysin and

naringenin are potent antioxidants and possess significant hepatoprotective against ALD. It is well documented that localization of flavonoids within the membranes may modify membrane fluidity and lipid peroxidation. Phenolic phytochemicals, due to their phenolic ring and hydroxyl substituents, can function as effective antioxidants by virtue of their ability to quench free radicals.[45] Quercetin, an isomer of morin seems to be a better antioxidant but it has lower bioavailability[46] and one of the main gratitude of morin is for its very minimal toxicity even at higher dose usage.[11] Furthermore, morin prevents mitochondrial depolarization and decline the release of apoptogenic factors from mitochondria by enhancing the expression of anti-apoptogenic factors like Bcl_2.[47] Hydroxyl group play an important role in antioxidant and anti-peroxidative properties rendered by morin.[48] Hence, the observed beneficial effects of morin attributes to its bioavailability and the presence of phenolic ring as well as five hydroxyl groups.

Histological observations under light microscopy revealed the presences of hepatocytic necrosis and inflammatory cells in the ethanol-fed rats. This could be due to the enhancement of lipid peroxidation as a result of lipids accumulation which forms a basis for cellular damage. Morin (60 mg and 120 mg/kg BW) reduces the severity of the damage in the hepatocytes. The above results demonstrate and support that morin preserves the structural integrity of the liver by virtue of its hepatoprotective, hypolipidemic, anti lipid peroxidative, antioxidant and properties.

5. CONCLUSION

Our findings demonstrated the beneficial effects of morin, a phytochemicals, in preventing the ethanol-induced hepatotoxicity. This might be due to its hepatoprotective, hypolipidemic, free radical scavenging and antioxidant effects. We believe that the low doses of morin (15 & 30 mg/kg BW) might not be sufficient to scavenge the radicals evidenced by histopathological examinations. Even though the other doses of morin i.e 60 mg and 120mg/kg BW have high potency in treating ethanol induced damages, there is no significant differences between there doses. Thus we conclude that the maximum efficacy is with the moderate dose of morin i.e 60mg/kg BW. Furthermore investigations are needed to study its molecular mechanism to understand its mode of action.

ACKNOWLEDGMENTS

This study was supported by the grants from The Science & Engineering Research Board (F.No.SR/FT/LS-135/2009), New Delhi, India. The authors are grateful to The Professor and Head, Department of Biochemistry and Biotechnology and the Principal, Rani Meyyammai College of Nursing, Annamalai University, Tamilnadu, India for providing the necessary amenities.

REFERENCE

1. Ho WY, Yeap SK, Ho CL, Abdul Rahim R, Alitheen NB (2012): Hepatoprotective activity of *Elephantopus scaber* on alcohol-induced liver damage in mice. *Evid Based Complement Alternat Med 2012*: 417953.
2. Liu S, Hou W, Yao P, Zhang B, Sun S, Nüssler AK, Liu L (2010): Quercetin protects against ethanol-induced oxidative damage in rat primary hepatocytes. *Toxicol In vitro 24*: 516-522.
3. Ashakumary L, Vijayammal PL (1993): Additive effect of alcohol and nicotine on lipid metabolism in rats. *Indian J Exp Biol 31*: 270-274.
4. Darwish HA, Naglaa R, Raboh A, Mahdy A (2012): Camel's milk alleviates alcohol-induced liver injury in rats. *Food Chem Toxicol 50*: 1377-1383.
5. Sen CK (2001): Antioxidant and redox regulation of cellular signalling: introduction. *Med Sci Sports Exerc 33(3)*: 368-370.
6. Sesso HD, Gaziano JM, Liu S, Buring JE (2003): Flavonoid intake and the risk of cardiovascular disease in women. *Am J Clin Nutr 77*: 1400-1408.
7. Smith C, Halliwell B, Aruoma OI (1992): Protection by albumin against the pro-oxidant actions of phenolic dietary components. *Food Chem Toxicol 30*: 483-489.
8. Kitagawa S, Sakamoto H, Tano H (2004): Inhibitory effects of flavonoidson free radical-induced hemolysis and their oxidative effects on hemoglobin. *Chem Pharm Bull 52*: 999-1001.
9. Al Numair KS, Chandramohan G, Alsaif MA, Baskar AA (2012): Protective effect of morin on cardiac mitochondrial function during isoproterenol-induced myocardial

infarction in male Wistar rats. *Redox Rep 17(1)*: 14-21.
10. Al-Numair KS, Chandramohan G, Veeramani C, Alsaif MA (2012): Morin, a flavonoid, prevents lysosomal damage in experimental myocardial ischemic rats. *J Med Plants Res 6(18)*: 3445-3449.
11. Prahalathan P, Kumar S, Raja B.(2012) Morin attenuates blood pressure and oxidative stress in deoxycorticosterone acetate-salt hypertensive rats: a biochemical and histopathological evaluation. *Metabolism* 61:1087–1099.
12. Sivaramakrishnan V, Niranjali Devaraj S (2010): Morin fosters apoptosis in experimental hepatocellular carcinogenesis model. *Chem Biol Interact 183*: 284-292.
13. Prahalathan P, Kumar S, Raja B (2012): Effect of morin, a flavonoid against DOCA-salt hypertensive rats: a dose dependent study. *Asn Pacific J Trop Biomed 2(6)*: 443-448.
14. Johnson D, Lardy H (1967): Isolation of liver and kidney mitochondria. In: Method in Enzymology. *Acadamic Press New York 10*: 94-96.
15. Lowry OH, Rosenbrough NJ, Parr AL (1951): Protein measurement with folin phenol reagent. *J Biol Chem 193*: 265-275.
16. Reitman S, Franke SA (1957): Colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvic transaminases. *Am J Clin Pathol 28*:56-63.
17. Kind PRN, King EJ (1954): Estimation of plasma phosphatases by determination of hydrolyzed phenol with aminoantipyrine. *J Clin Path 7*: 330-322.
18. Rosalki SB, Rau D (1972): Serum gamma-glutamyl transpeptidase activity in alcoholism. *Clin Chim Acta 39*: 41-47.
19. Malloy HT, Evelyn KA (1937): The determination of bilirubin with the photoelectric colorimeter. *J Bio Chem 119*: 48-490.
20. Folch J, Lee M, Stanley GHS (1957): A simple method for the isolation and purification of total lipid from animal tissue. *J Biol Chem 226*: 497-509.
21. Zlatkis A, Zak B, Boyle GJ (1953): A method for the determination of serum cholesterol. *J Clin Med 41*: 486-492.
22. Foster LB, Dunn RT (1973): Stable reagents for determination of serum triglycerides by colorimetric hantzsch condensation method. *Clin Chem 19*: 338-340.
23. Falholt K., Falholt W., Lund B (1973): An easy colorimetric method for routine determination of free fatty acids in plasma. *Clin Chim Acta 46*: 105-111.
24. Zilversmit DB, Davis AK (1950): Microdetermination of plasma phospholipids by means of precipitation with trichloroacetic acid. *J Lab Clin Invest 35*: 155-160.
25. Izzo C, Grillo F, Murador E (1981): Improved method for the determination of high density lipoprotein cholesterol. *Clin. Chem 27:* 371-374.
26. Friedwald WT, Levy RI, Fredrickson DS (1981): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem 18*: 499-502.
27. Slater EC, Bonner WD (1952): Effect of fluoride on succinate oxidase system. *J Biochem 52*: 185-196.
28. Niehaus WG, Samuelsson B (1968): Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem 6:*126-130.
29. Jiang ZY, Hunt JV, Wolff SP (1992): Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Anal Biochem 202*: 384-389.
30. Saravanan N, Nalini N (2007): Antioxidant effect of *Hemidesmus indicus* on ethanol-induced hepatotoxicity in rats. *J Med Food 10(4)*: 675-682.
31. Saravanan N, Rajasankar S, Nalini N (2007): Antioxidant effect of 2-hydroxy-4-methoxy benzoic acid on ethanol-induced hepatotoxicity in rats. *J Pharm Pharmacol 59(3)*: 445-453.
32. Lee HS, Jung KH, Hong SW, Park IS, Lee C, Han HK, Lee DH, Hong SS (2008).Morin protects acute liver damage by carbon tetrachloride (CCl(4)) in rat. *Arch Pharm Res* 31(9):1160-5.
33. Rajasree CR, Rajamohan T, Augusti KT (1999): Biochemical effects of garlic protein on lipid metabolism in alcohol fed rats. *Indian J Exp Biol 37(3)*: 243-247.
34. Prahalathan P, Saravanakumar M, Raja B (2012): The flavonoid morin restores

blood pressure and lipid metabolism in DOCA-salt hypertensive rats. *Redox Rep 17(4)*: 167-175.
35. Pushpakiran G, Mahalakshmi K, Viswanathan P, Anuradha CV (2005): Taurine prevents ethanol-induced alterations in lipids and ATPases in rat tissues. *Pharma Reports 57*: 578-587.
36. Garcia-Villafranca JG, Guillen A, Castro J (2008): Ethanol consumption impairs regulation of fatty acid metabolism by decreasing the activity of AMP-activated protein kinase in rat liver. *Biochimie 90*: 460–464.
37. Hannuksela ML, Ramet ME, Nissinen AET, Liisanantti MK, Savolainen MJ (2003): Effects of ethanol on lipids and atherosclerosis. *Pathophysiol 10*: 93-103.
38. Walldius G, Jungner I (2006): The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy-a review of the evidence. *J Intern Med 259(5)*: 493-519.
39. Karthikesan K, Pari L (2008): Caffeic acid alleviates the increased lipid levels of serum and tissues in alcohol-induced (hepatotoxicity in) rats. *Fundam Clin Pharmacol 22(5)*: 523-527.
40. Setshedi M, Wands JR, Suzanne M, Monte DL (2010): Acetaldehyde adducts in alcoholic liver disease. *Oxid Med Cell Longev 3(3)*: 178-185.
41. Baraona E, Di Padova C, Tabasco J, Lieber CS (1987): Red blood cells: a new major modality for acetaldehyde transport from liver to other tissues. *Life Sci 40*:253–258.
42. Bansal S, Srinivasan S, Anandasadagopan S, Chowdhury AR, Selvaraj V, Kalyanaraman B, Joseph J, Avadhani NG (2012): Additive effects of mitochondrion-targeted cytochrome CYP2E1 and alcohol toxicity on cytochrome c oxidase function and stability of respirosome complexes. *J Biol Chem 287(19)*: 15284-15297.
43. Lakshmi Devi S, Anuradha CV (2010): Mitochondrial damage, cytotoxicity and apoptosis in iron-potentiated alcoholic liver fibrosis: amelioration by taurine. *Amino Acids 38(3)*:869-879.
44. Senthilkumar R, Sengottuvelan M, Nalini N (2004): Protective effect of glycine supplementation on the levels of lipid peroxidation and antioxidant enzymes in the erythrocyte of rats with alcohol-induced liver injury. *Cell Biochem Funct 22(2)*: 123-128.
45. Wang L, Tu YC, Lian TW, Hung JT, Yen JH, Wu MJ (2006): Distinctive antioxidant and antiinflammatory effects of flavonols. J Agric Food Chem 54(26): 9798-9804.
46. Hou YC, Chao PD, Ho HJ, Wen CC, Hsiu SL (2003): Profound difference in pharmacokinetics between morin and its isomer quercetin in rats. *J Pharm Pharmacol 55(2)*: 199-203.
47. Kapoor R, Kakkar P (2012): Protective role of morin, a flavonoid, against high glucose induced oxidative stress mediated apoptosis in primary rat hepatocytes. *PLoS One* 7(8): e41663.
48. Zhang R, Kang KA, Kang SS, Park JW, Hyun JW (2011): Morin (2,3,4′,5,7 - pentahydroxyflavone) protected cells against γ-radiation-induced oxidative stress. *Basic Clin Pharmacol Toxicol 108*: 63–72.