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Efficacy of morin on ethanol-induced oxidative stress in rat intestine

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Abstract: Ethanol is known to induce oxidative stress in the intestine and initiates gastrointestinal tract disorders. This study was evaluated the protective effect of morin on ethanol-induced oxidative stress in the intestine of rats. Administering ethanol (6 g/ kg BW) to rats for the period of 60 days resulted in significant elevated levels of intestinal lipid peroxidation by products such as thiobarbituric acid reactive substance (TBARS), lipid hydro peroxide (LOOH) and lipid levels (Cholesterol, Triglycerides, Phospholipids and Free Fatty acid). Moreover ethanol-fed rats showed lowered activities/level of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and reduced glutathione (GSH) as compared with those of normal control rats. Ethanol fed rats treated with morin (60mg/kg BW) daily for a period post 30 days significantly ($p < 0.05$) modulates the enzymic antioxidants, reduced lipid peroxidation and lipid levels as compared to untreated ethanol fed rats. In conclusion, morin can effectively protected the intestine against ethanol-induced oxidative stress by directly enhancing the levels of endogenous antioxidants, reducing the levels of lipid peroxidation and lipids

Introduction

Alcohol consumption is considered to be a serious public health problem, and it is responsible for 3.2% of mortality worldwide. According to the World Health organisation (WHO) alcoholic liver disease (ALD) is one of the major causes of illness and death worldwide [1]. Recent studies showed that disruption of intestinal barrier function by alcohol causes increase in intestinal permeability to macromolecules as evidenced by both clinical and experimental studies [2]. Therefore, the action of alcohol at the level of gastrointestinal mucosa appears to be the first site of injury that leads to the development of a complex [3]. The intestinal barrier is the most important biological barrier against the toxic substance. The direct contact of ethanol with the GIT may elicit several metabolic changes among which is the induction of ethanol-inducible cytochrome P450 (CYP2E1) with an attendant generation of toxic acetaldehyde and reactive oxygen species (ROS) [4]. These metabolites are capable of depleting endogenous antioxidant status and may thus disturb the integrity intestinal membrane [5]. Diet may exert multiple protective biological effects on the mucosa of the gastrointestinal tract. Fruits and vegetables, in fact, seem to play a preventive role against the development of gastric erosions or gastro or cyto-protection[6]. On the basis of these considerations, the aim of the present study was to evaluate the protective effect of morin on ethanol-induced oxidative stress in the intestine. Morin, a natural bioflavonoid present in Almond (*Prunus dulcis*) [7], fig (*Chlorophora tinctoria*) [8], *Psidium guajava* (Indian guava) and Moraceae family, which have been using in traditional herbal medicine [9]. Morin exhibits antioxidant [10], anti-inflammatory [11], hepatoprotective [12], antiproliferative [13], modulate cyclo-oxygenase activity [14] and used as a food preservative [15]. In this study morin was administered during ethanol intoxication in order to determine its effects on ethanol-induced oxidative injury in the intestine. Our hypothesis is that the use of herbal medicine may make it possible to alleviate the incidence of alcohol induced gastrointestinal disorders via inhibition of oxidative stress.

Materials and Methods

Animals

Male albino wistar rats (150-180 g) were procured from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India and maintained in an air conditioned room with a 12 h

light/12 dark cycle. Feed and water were provided *ad libitum*. All the experimental studies were conducted in the Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, in accordance with the National Institute of Health of Guide for the Care and Use of Laboratory Animals (NIH 1985). The experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital, Annamalainagar. (Reg No: 166/1999/CPCSEA)

Drug and Chemicals

Morin hydrate was purchased from Sigma Chemicals Co., St. Louis, Mo, USA. Absolute ethanol used in our study was obtained from Cuddalore District, South India. Thiobarbituric acid was purchased from Sigma Aldrich (P) Ltd., Mumbai, India. 5,5'-Dithiobis (2-nitro benzoic acid), phenazine methosulphate and reduced nicotinamide adenine dinucleotide (NADH) were purchased from Sisco Research Laboratories, Pvt., Mumbai, India. Glutathione was obtained from S.D. Fine Chemicals, Mumbai, India. All the other chemicals used were of analytical grade and the organic solvents were distilled before use.

Suspension of the interested compound and mode of administration

Morin was freshly solubilised in water [16] and was administered to rats orally using an intragastric tube daily for a period post 30 days.

Experimental Design

The animals were divided into four groups of six rats each and were maintained as follows

- Group I: Normal control rats, received isocaloric glucose from a 40% stock solution twice in a day, which was isocaloric to ethanol.
- Group II: Rats received isocaloric glucose from a 40% stock solution every day and aqueous solution of morin (60mg/ kg BW) via intubation.
- Group III: Rats received ethanol (6g/kg BW) from a 30% stock solution twice in a day for a period of 60 days.
- Group IV: Rats received ethanol (6g/kg BW) and co-treated with morin (60 mg/kg BW) from the 30th day along with ethanol.

Collection and Preparation of Tissue

The total duration of the experiment was 60 days, at the end of which, the animals were anaesthetised using light ether and killed by cervical decapitation. The proximal portion (15cm) of the small intestine was carefully dissected, washed and flushed thoroughly with ice-cold physiological saline, blotted on filter paper, weighed, and homogenized in ice-cold 1.15% KCl (1g/3ml) at 5000 rpm for 15min at 4°C. Aliquots of the supernatant was used for the following estimations.

Biochemical estimations

Lipid peroxidation by products such as thiobarbituric acid reactive species (TBARS) was hydroperoxides (LHP) were measured by the method of Niehaus and Samuelson [17] and Jiang et al. [18]. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and reduced glutathione (GSH) by the methods previously described elsewhere[19]. Protein content of tissues was by method of Lowry et al [20]. Extraction of lipid was by the method of Folch et al. [21]. The content cholesterol and triglycerides (TGs) were assessed according to methods of Zlatki et al. [22] and Foster and Dunn [23]. Phospholipids (PL) were assayed by the method of Zilversmit and Davis [24].

Statistical analysis

All the grouped data were evaluated statistically and the significant changes caused by the treatment was determined using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) by using SPSS 17 for Windows. The results are expressed as means \pm SD of six rats from each group. The level of statistical significance was set at $p < 0.05$.

Results and Discussion

Table 1 shows the average weight gained by the animals during the total experimental period of 60 days. The weight gain was significantly reduced in ethanol-fed rats as compared to the control animals. Ethanol – fed treated rats co-treated with morin significantly increased the weight gain. Morin alone treated to showed no alteration in control rats.

Table.1 Effect of morin on body weights changes on control and experimental animals

Groups	Initial body weight (g)	Final body weight (g)	weight gain (g)
CON	155.20 \pm 10.30 ^a	220 \pm 9.70 ^a	64.80 \pm 5.45 ^a
Con + M (60 mg /kg BW)	150.15 \pm 8.90 ^a	213.45 \pm 12.65 ^a	63.30 \pm 4.20 ^a
EtOH	160.10 \pm 12.25 ^a	192.15 \pm 14.50 ^b	32.05 \pm 2.15 ^b
EtOH + M (60 mg /kg BW)	157. 70 \pm 11.10 ^a	205.15 \pm 17.24 ^a	47.45 \pm 3.65 ^c

CON- Control; CON+ M- Control +Morin;EtOH – Ethanol;EtOH+M – Ethanol+Morin

Values are means \pm SD for six rats: Values not sharing a common superscript differ significantly at $p < 0.05$.ANOVA followed by Duncan's Multiple Range Test (DMRT)

Table 2 shows the effect of morin on the levels of lipid peroxidation by products and Glutathione in the intestine of control and experimental

Table. 2 shows the level of TBARS and LOOH in normal and experimental animals

Groups	TBARS (mM/g tissue)	LHP (μ M/ mg tissue)	Glutathione (mM / g tissue)
CON	0.61 \pm 0.04 ^a	0.34 \pm 0.02 ^a	9.85 \pm 0.72 ^a
CON + M (60 mg /kg BW)	0.58 \pm 0.03 ^a	0.27 \pm 0.02 ^a	9.90 \pm 0.64 ^a
EtOH	2.29 \pm 0.18 ^b	3.05 \pm 0.31 ^b	6.88 \pm 0.56 ^b
EtOH + M (60 mg /kg BW)	1.15 \pm 0.12 ^c	0.79 \pm 0.05 ^c	8.25 \pm 0.77 ^c

CON- control; CON+ M – control+ Morin; EtOH – Ethanol; EtOH +M- Ethanol + Morin

Values are means \pm SD for six rats:

Values not sharing a common superscript differ significantly at $p < 0.05$. NOVA followed by Duncan's Multiple Range Test (DMRT) : units : TBARS (mM/g tissue), LHP (μ M/ mg tissue), Glutathione (mM / g tissue)

Table 3 shows the effect of oral administration of morin on the levels of enzymatic antioxidants viz SOD, CAT and GPx in control and experimental animals. Ethanol-fed rats showed decreased activities of SOD, CAT and GPx. whereas the administration of morin significantly increased these enzyme activities when compared to the untreated ethanol-fed rats.

Table. 3 Effect of morin on the levels of enzymic antioxidants in the intestine

Groups	U/mg protein		
	SOD	CAT	GPx
CON	6.61 \pm 0.42 ^a	58.55 \pm 3.42 ^a	0.59 \pm 0.04 ^a
Con + M (60 mg /kg BW)	6.83 \pm 0.52 ^a	59.27 \pm 4.48 ^a	0.61 \pm 0.05 ^a
EtOH	3.25 \pm 0.21 ^b	37.61 \pm 3.51 ^b	0.33 \pm 0.03 ^b
EtOH + M (60 mg /kg BW)	5.35 \pm 0.42 ^c	44.83 \pm 4.20 ^c	0.48 \pm 0.02 ^c

CON- Control; CON+ M- Control +Morin;EtOH – Ethanol;EtOH+M – Ethanol+Morin

Values are means \pm SD for six rats: Values not sharing a common superscript differ significantly at $p < 0.05$.ANOVA followed by Duncan's Multiple Range Test (DMRT): Units: SOD: Enzyme concentration required to inhibit 50% of chromogen/ min/ mg protein; CAT: μ M of H_2O_2 decomposed /min/mg protein;GPx: μ M of NADPH Oxidized/min/mg Protein

Table 4 shows the effect of morin on the levels of lipids in normal control and experimental animals. Significant elevated levels of lipids were observed in ethanol-fed. Oral administration of morin to the ethanol-fed rats significantly the dyslipidimia when compared to the untreated rats.

Table 4. Effect of morin on the levels of lipids in the intestine of control and experimental animals

Groups	μ g / mg tissue		
	Total cholesterol	Triglycerides	Phospholipids
CON	4.15 \pm 0.32 ^a	3.61 \pm 0.33 ^a	6.80 \pm 0.40 ^a
Con + M (60 mg /kg BW)	4.10 \pm 0.40 ^a	3.52 \pm 0.30 ^a	6.73 \pm 0.59 ^a
EtOH	7. 80 \pm 0.42 ^b	6.61 \pm 0.55 ^b	10.35 \pm 1.11 ^b
EtOH + M (60 mg /kg BW)	5.30 \pm 0.42 ^c	4.18 \pm 0.42 ^c	7.75 \pm 0.60 ^c

CON- Control; CON+ M- Control +Morin;EtOH – Ethanol;EtOH+M – Ethanol+Morin Values are means \pm SD for six rats: Values not sharing a common superscript differ significantly at $p < 0.05$.ANOVA followed by Duncan's Multiple Range Test (DMRT)

Discussion

Chronic ethanol consumption can exert deleterious effects on the structures and functions of all parts of the Gastro intestinal tract (GIT) via oxidative stress [25]. This may interfere with digestion and absorption of essential nutrients in the GIT [26]. Poor nutritional status, as a consequence of alcohol-induced primary and secondary malnutrition, has been reported to contribute to loss in body weight and the development of other alcohol related pathologies [27]. Hence, in our study, there was a significant increase in body weight gain in all animals except the Ethanol alone fed treated group..Treatment with morin significantly restored body weight gain. It might be due the gastrointestinal

productive effect of morin through its antioxidant, antiinflammatory activities or related to increasing appetite and better utilization of nutrients in the diet, leading to increases in body weight. Earlier, it was demonstrated that, in the intestine, the reactions of ethanol and acetaldehyde catabolism were mediated by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) present in the gastric and intestinal mucosae [28]. An increase in ethanol oxidation by small intestinal microsomes [29] and an induction of CYP2E1 in rat colon after alcohol feeding [30] were reported. MEOS activity in the GIT contributes to ethanol oxidation in order to produce acetaldehyde and free radicals playing an important role in the pathogenesis of various alcoholic injuries [31]. Oxidative stress and depletion of anti-oxidants have also been considered a crucial step in alcohol-induced mucosal damage. One of the major mechanisms suggested to underlie the induction of gastric erosions by absolute alcohol is the oxidative damage with its dual events of lipid peroxidation and oxygen reactive species generation. Actually, oxygen-derived radicals have been implicated in the pathogenesis of gastric tissue damage [32].

The concept that anti-radicals could protect against alcohol-induced gastric injury has been established in a number of studies. Lipid lowering agent may protect against gastric mucosal injury and promote the healing of chronic gastric ulcers by its anti-oxidant activity and their radical scavenging activities [33]. Enhanced oxidative stress production in interstitial tract leads to the formation of peroxidation of membrane phospholipids. The peroxidation of membrane phospholipids is basically damage because the formation of lipid peroxidation products leads to the spread free radicals and cytotoxic aldehyde by products. In our present study, there was a marked increased in the level of lipid peroxidation by products in the intestine of ethanol-fed animals relative to control animals. Epithelial mucosa cell integrity can be protected from deleterious effects of ROS by antioxidant defence system consisting of nonenzymic antioxidants (glutathione (GSH), Vitamins A,C and vitamin E)and enzymic antioxidants such superoxidase dismutase (SOD),catalase and glutathione peroxidase (GPx) [34]. Flavonoids and other polyphenol derived plant materials with potential beneficial effects on human health. Over the years they have found to be an important part of human diet and are considered to be active principle in some medicinal plants. The activity of flavonoids is efficient in trapping superoxide anion, hydroxyl, peroxy and alcohoxyl radicals. Moreover, they have membrane stabilizing property and also inhibit lipid peroxidation some of them have been shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa showing cytoprotective effects [35]. Morin treatment facilitates the recovery of the damaged tissue in the chronic phase of TNBS induced rat colitis, an effect associated with an amelioration in the production of some of the mediators involved in the inflammatory response of the intestine, such as free radicals, leukotriene B4, nitric oxide and interleukin [36]. In conclusion, the mechanism by which morin protects intestinal barrier from alcohol injury is not fully established. However, two possibilities can be addressed. Firstly, morin may exert a beneficial action on the intestinal antioxidants, secondly; the effect of morin is partly due to its antiinflammatory activities.

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