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# Protective effect of morin on lipid peroxidation and lipid profile in ammonium chloride-induced hyperammonemic rats

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ABSTRACT

**Objective:** To evaluated the protective effects of morin (3, 5, 7, 2′, 4′-pentahydroxyflavone) on lipid peroxidation and lipid levels during ammonium chloride (AC) induced hyperammonemia in experimental rats. **Methods:** Thirty two male albino Wistar rats, which are weighing between 180-200 g were used for the study. The hyperammonemia was induced by administration of 100 mg/kg body weight (*i.p.*) thrice in a week of AC for 8 weeks. Rats were treated with morin at dose (30 mg/kg body weight) via intragastric intubations together with AC. At the end of experimental duration, blood ammonia, plasma urea, lipid peroxidation indices [thiobarbituric acid reactive substances, hydroperoxides and lipid levels (cholesterol, triglycerides, free fatty acids and phospholipids)] in serum and tissues were analysed to evaluate the antiperoxidative and antilipidemic effects of morin. **Results:** Ammonia, urea, lipid peroxidative indices and lipid levels were significantly increased in AC administered group. Morin treatment resulted in positive modulation of ammonia, urea, lipid peroxidative indices and lipid levels. Morin administration to normal rats did not exhibit any significant changes in any of the parameters studied. **Conclusions:** It can be concluded that the beneficial effect of morin on ammonia, urea, lipid peroxidative indices and lipid levels could be due to its antioxidant property.

## 1. Introduction

In living organisms, ammonia is an important nitrogen substrate in several reactions, and plays an important role in nitrogen homeostasis of cells. Moreover, it is a product as well as precursor of various important nitrogen containing metabolites such as amino acids, which in turn are the smallest subunits of proteins. Hyperammonemia is a major contributing factor to neurological abnormalities observed in hepatic encephalopathy and in congenital defects of ammonia detoxication. Ammonia affects both excitatory and inhibitory synaptic transmission in the mammalian brain by a variety of mechanisms[1]. Ammonia toxicity occurs partly via oxidative stress, which leads to lipid peroxidation and free radical generation. This causes hepatic dysfunction and failure, which is a primary cause of neurological disorders and alterations in the function of the central nervous system associated with hyperammonemia, such as, hepatic encephalopathies, Reye syndrome, irritability, somnolence, vomiting, seizures, and derangement of cerebral function, coma and death[2-4].

The greatest disadvantage of presently available potent conventional or synthetic antihyperammonemic agents/therapies lies in their toxicity and reappearance of symptoms after discontinuation. Furthermore, these drugs can cause serious adverse effects[5]. Hence, the screening and development of therapeutic agents from traditional medicinal plants (active components) for their antihyperammonemic activity is in progress worldwide. Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. They are widely distributed in foods of plant origin such as vegetables, fruits, tea and wine. Scavenging of free radicals seems to play a considerable part of the antioxidant activity of flavonoid compounds. Regular ingestion of flavonoid-containing foods may protect against the adverse effect of ammonia toxicity. Phytopharmaceuticals are gaining importance in allopathic as well as in traditional medicine owing to their non-addictive and non-toxic nature. Novel antioxidants may offer an effective and safe means of counteracting some of the problems and bolster the body′s defenses against free radicals and hyperammonemia.

Morin (3, 5, 7, 2′, 4′ -pentahydroxyflavone) is a kind of flavonoid belonging to the group of flavonols (Figure 1), found in old fustic (*Chlorophora tinctoria*), osage orange

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(*Maclura pomifera*), almonds (*P. guajava* L.), mill (*Prunus dulcis*), fig (*Chlorophora tinctoria*), onion and apple and other moraceae which are used as dietary agents and also as herbal medicines. It has been shown to be act as a potent antioxidant and metal ion chelating capacities, possesses various biological and biochemical effects including antioxidation, anti-mutagenesis, anti-inflammation, cardioprotective activities, anticancer, xanthine oxidase inhibitor, protein kinase C inhibitor and cell proliferation inhibitor[6-8].

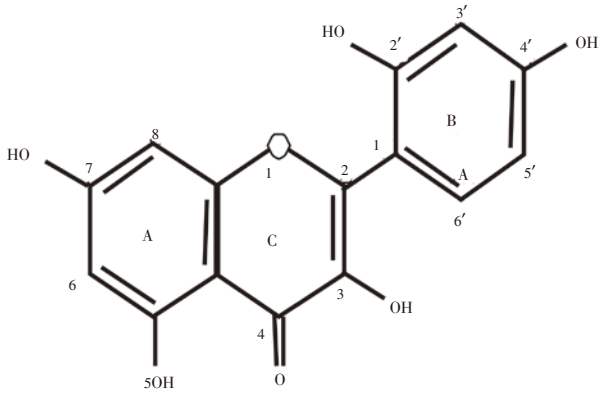


**Figure 1.** Structure of morin.

Further, morin is a well known therapeutic agent for a number of diseases. To our knowledge, this report is the first study to investigate the effect of morin on lipid peroxidation and lipid levels in ammonium chloride (AC) induced hyperammonemic rats. Therefore the objective of the present study is to investigate the influence of morin on blood ammonia, plasma urea and lipid peroxidation induces [thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP)) as well as serum and tissues lipid levels (cholesterol, triglycerides, free fatty acids and phospholipids)] in an animal model of AC induced hyperammonemia.

## 2. Materials and methods

### 2.1. Experimental animals

All the experiments were carried out with male albino Wistar rats weighing 180-200 g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47 cm×34 cm×20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22 ℃ and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Approval No. 424; dated 21/03/2007).

### 2.2. Drugs and chemicals

Morin, thiobarbituric acid and butylated hydroxy toluene were purchased from Sigma Chemical Company, St. Louis, MO, USA. AC was purchased from Sisco Research Laboratories, Mumbai, India. All other biochemicals and chemicals used in the study were of analytical grade.

### 2.3. Preparation of morin

Morin was freshly dissolved in small amount of ethanol and then diluted with physiological saline[5].

### 2.4. Induction of experimental hyperammonemia

Hyperammonemia was induced in Wistar rats by intraperitoneal injections of ammonium chloride at a dose of 100 mg/kg body weight thrice in a week for 8 consecutive weeks[9].

### 2.5. Experimental design

In the experiment, a total of 32 rats were used. The rats were divided into four groups of 8 rats each. Group I: rats received physiological saline and considered as control; Group II: rats were orally administered with morin (30 mg/kg) using an intragastric tube[10]; Group III: rats treated with AC (100 mg/kg; *i.p.*); Group IV: rats treated with AC + morin thrice in a week for 8 weeks.

At the end of the 8 weeks, rats were fasted overnight and sacrificed by cervical decapitation. Blood samples were collected for various biochemical estimations such as blood ammonia, plasma uric acid, TBARS, HP, serum and tissues cholesterol, triglycerides, free fatty acids and phospholipids[11-18]. Liver, kidney and brain were dissected out, washed using chilled saline and used for lipid estimations.

### 2.6. Statistical analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) by using the statistical package of social sciences (SPSS) 10.0 for Windows. The significance level was $P<0.05$.

## 3. Results

Table 1 and 2 shows the levels of blood ammonia, plasma urea, TBARS, HP and serum lipids levels (cholesterol, triglycerides, free fatty acids and phospholipids) of control and experimental animals. Hyperammonemia indicated by ammonia, uric acid, lipid peroxidation indicated by TBARS, HP and serum lipid levels were significantly higher in AC administered animals as compared to those of normal control rats. Ammonia, uric acid, TBARS, HP and serum lipid levels were lowered significantly in AC administered animals treated with morin.

Table 3 shows the activities of lipid levels (cholesterol, triglycerides, free fatty acids and phospholipids) in tissues (liver, kidney and brain) of control and experimental animals. Cholesterol, triglycerides, free fatty acids and phospholipids activities in tissues of rats on AC administered rats were significantly higher than the control rats. Treatment with the morin to AC administered rats significantly reduced cholesterol, triglycerides, free fatty acids and phospholipids activities as compared to those animals on AC treatment alone.

**Table 1**
Effect of morin on changes in the blood ammonia, plasma urea, TBARS, and HP of normal and experimental rats (Mean ± SD).

| Groups | Blood ammonia (μ mol/L) | Plasma urea (mg/dL) | TBARS (nmol/mL) | HP ($\times10^{-5}$mmol/dL) |
|---|---|---|---|---|
| Normal | 84.80 ± 5.27[a] | 9.95 ± 0.62[a] | 2.78 ± 0.21[a] | 8.10 ± 0.62[a] |
| Morin (30 mg/kg) | 78.47 ± 4.95[a] | 9.06 ± 0.57[a] | 2.95 ± 0.22[a] | 8.24 ± 0.54[a] |
| AC treated (100 mg/kg) | 374.46 ± 22.10[b] | 23.67 ± 1.40[b] | 4.62 ± 0.35[b] | 13.14 ± 1.01[b] |
| AC + morin (30 mg/kg) | 147.87 ± 10.80[c] | 12.66 ± 0.92[c] | 3.38 ± 0.26[c] | 9.48 ± 0.73[c] |

Values not sharing a common superscript letter differ significantly ($P<0.05$).

**Table 2**
Changes in the levels of serum lipid profile in normal and experimental rats (Mean ± SD).

| Groups | Cholesterol (mg/dL) | Triglycerides (mg/dL) | Free fatty acids (mg/dL) | Phospholipids (mg/dL) |
|---|---|---|---|---|
| Normal | 86.01 ± 5.57[a] | 60.13 ± 4.58[a] | 74.24 ± 4.80[a] | 102.37 ± 7.79[a] |
| Morin (30 mg/kg) | 81.03 ± 6.17[a] | 54.96 ± 3.56[a] | 70.13 ± 5.34[a] | 99.98 ± 6.47[a] |
| AC treated (100 mg/kg) | 172.32 ± 13.19[b] | 92.70 ± 7.10[b] | 151.32 ± 11.58[b] | 172.34 ± 13.19[b] |
| AC+ morin (30 mg/kg) | 123.69 ± 9.45[c] | 71.55 ± 5.46[c] | 91.15 ± 6.96[c] | 122.70 ± 8.05[c] |

Values not sharing a common superscript letter differ significantly ($P<0.05$).

**Table 3**

| Lipid profile in tissue | | Normal | Morin (30 mg/kg) | AC treated (100 mg/kg) | AC + morin (30 mg/kg) |
|---|---|---|---|---|---|
| Cholesterol (mg/100 g tissue) | Liver | 335.23 ± 25.53[a] | 329.89 ± 22.93[a] | 482.42 ± 33.53[b] | 377.49 ± 28.74[c] |
| | Kidney | 443.65 ± 33.78[a] | 435.39 ± 33.15[a] | 704.77 ± 53.67[b] | 505.42 ± 38.49[c] |
| | Brain | 1459.06 ± 101.42[a] | 1427.38 ± 99.21[a] | 2692.73 ± 187.17[b] | 1807.76 ± 125.66[c] |
| Triglycerides (mg/100 g tissue) | Liver | 316.81 ± 24.12[a] | 305.00 ± 23.22[a] | 517.33 ± 39.39[b] | 378.64 ± 28.83[c] |
| | Kidney | 458.81 ± 34.94[a] | 441.25 ± 33.60[a] | 623.57 ± 47.48[b] | 531.65 ± 40.48[c] |
| | Brain | 327.49 ± 24.94[a] | 314.68 ± 23.96[a] | 495.85 ± 37.76[b] | 377.90 ± 28.78[c] |
| Free fatty acids (mg/100 g tissue) | Liver | 734.74 ± 55.95[a] | 726.23 ± 55.30[a] | 1256.97 ± 95.71[b] | 821.57 ± 62.56[c] |
| | Kidney | 795.56 ± 60.58[a] | 778.00 ± 59.24[a] | 1674.51 ± 116.39[b] | 934.48 ± 71.16[c] |
| | Brain | 13.54 ± 1.03[a] | 12.18 ± 0.93[a] | 33.46 ± 2.55[b] | 18.26 ± 1.39[c] |
| Phospholipids (mg/100 g tissue) | Liver | 1165.43 ± 81.01[a] | 1157.88 ± 80.48[a] | 2681.68 ± 186.40[b] | 1451.91 ± 100.92[c] |
| | Kidney | 1080.71 ± 75.12[a] | 1058.18 ± 73.55[a] | 2238.91 ± 155.62[b] | 1278.56 ± 88.87[c] |
| | Brain | 2168.54 ± 150.73[a] | 2140.03 ± 148.75[a] | 3031.86 ± 210.74[b] | 2413.72 ± 167.78[c] |

Values not sharing a common superscript letter differ significantly ($P<0.05$).

## 4. Discussion

Ammonia is present in all living organisms as a product of degradation of proteins and other nitrogenous compounds. However, at higher levels, ammonia is toxic, leading to functional disturbances in the central nervous system that could lead to coma and death. To avoid the deleterious effects of ammonia, ureotelic animals detoxify ammonia by incorporating it into urea that is eliminated in urine. However, when the liver fails, or when blood is shunted past the liver, blood ammonia levels elevated and brain function deteriorates[19]. In the liver, ammonia is removed either in the form of urea in periportal hepatocytes and/or as glutamine in perivenous hepatocytes[20]. Increased levels of circulatory ammonia and urea might indicate a hyperammonemic condition in rats treated with AC[4], which may be due to liver damage caused by ammonia intoxication. Administration of morin to AC induced hyperammonemic rats significantly decreased the levels of blood ammonia and urea. The reduction in the levels of ammonia and urea during morin treatment shows the potent anti-hyperammonemic effect of morin[10].

In our study, the elevated levels of lipid peroxidation induces in AC treated rats might be due to the liver damage caused by ammonia-induced free radical generation. Reports have shown that excess ammonia intoxication leads to excessive activation of N-methyl-Daspartate (NMDA) receptors leading to neuronal degeneration and death[9]. The mechanisms by which excessive activation of NMDA receptors leads to neuronal degeneration and death are caused by increased $Ca^{2+}$ concentration in the postsynaptic neuron. $Ca^{2+}$ binds to calmodulin and activates nitric oxide synthase, increasing the formation of nitric oxide (NO) that contributes to the neurotoxic process. Activation of NMDA receptors also leads to increased production of superoxide radical, which has been also proposed under *in vivo* conditions[4]. Superoxide and NO have the ability to generate hydroxyl radicals. This leads to oxidative stress, which causes tissue damage[9]. Decreased levels of circulatory lipid peroxidation products in morin administered rats may be due to its free radical scavenging property as it is an effective free radical scavenger[19,20]. Previous studies show that morin offers neuroprotection by inhibiting excess activation of NMDA receptors and NMDA receptor mediated neurotoxicity. The protect potent neuroprotective activity of morin could be of therapeutic value for the treatment of acute neuronal damage and disability[21].

In the present study, the administration of AC caused significant increase in the levels of lipid levels in serum and tissues (cholesterol, triglycerides, phospholipids and free fatty acids). It has been reported that AC could deplete the levels of α-KG and other Krebs cycle intermediates[2] and this could elevate the levels of acetyl CoA. This acetyl CoA may be used for the synthesis of fatty acids and cholesterol,

since fatty acids of different sources are used as substrates for synthesizing triglycerides and phospholipids. The elevated levels of acetyl CoA may increase levels of lipid profile (free fatty acids, triacylglycerols, phospholipids and cholesterol), as observed in our study. Another important function of α-KG occurs in the formation of carnitine[2]. Carnitine acts as a carrier of fatty acids into cell mitochondria so that proper catabolism of fats can proceed. The decreased α-KG and other Krebs cycle intermediates levels in rats treated with AC might have led to accumulation of fatty acids[22].

Lowering of serum/tissue lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of various vascular diseases. In the present study, morin treatment to hyperammonemic rats caused a significant decrease in serum and tissue lipids (cholesterol, triglycerides, phospholipids and free fatty acids). The effect of morin on controlled mobilization of serum cholesterol, triglycerides, phospholipids and free fatty acids presumably mediated possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. Phenolic compounds have the ability to normalize the levels of serum and tissue lipids during diseased conditions. It was reported that the morin possess both antioxidant effects and hypolipidemic effects against low density lipoprotein oxidation *in vivo*[6]. Therefore, it is possible mechanisms by which the morin modulates lipid peroxidation and lipid levels during hyperammonemic condition could be attributed to the natural antioxidants, inhibiting excess activation of NMDA receptors, ammonia lowering effect and enhancing the proper metabolism of fats, which could suppress oxygen radical generation and prevent lipid peroxidative damage in rats. But the exact mechanism is still unclear and further research on the action of morin is underway.

The biochemical findings obtained from our study indicates that morin exerts protection to ammonium chloride-induced hyperammonemic rats against oxidative stress and lipids. This could be due to prevention or inhibition of lipid peroxidative system by its antioxidant. In summary, morin has been shown to possess antihyperlipidemic effect in ammonium chloride-induced hyperammonemic rats by means of ammonia detoxication and antioxidant properties. But the exact mechanism is still unclear and further research is needed.

## Conflict of interest statement

We declare that we have no conflict of interest.

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