Effect of naringin on ammonium chloride-induced hyperammonemic rats: A dose-dependent study

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Abstract

Introduction: In mammals, ammonia is primarily a toxin that affects the central nervous system, leading to liver damage and urea cycle disorder. Increased accumulation of ammonia results in a spectrum of effects on the central nervous system exerted through both excitatory and inhibitory neurotransmission. The aim of this investigation was to examine the protective effect of naringin on ammonium chloride (NH4Cl)-induced hyperammonemic rats.

Materials and methods: Male albino Wistar rats were induced with intraperitoneal injections of NH4Cl [100 mg/kg body weight (b.w.)] and were treated with different concentrations of naringin (40 mg/kg, 80 mg/kg, and 160 mg/kg of b.w.) 3 times/wk for 8 consecutive weeks.

Results: Hyperammonemic rats showed elevated levels of ammonia, uric acid, creatinine, and bilirubin, and a reduced level of plasma urea. Body weights of normal and experimental rats were also measured. Oral administration of naringin significantly decreased the levels of blood ammonia, uric acid, creatinine, and bilirubin, and drastically increased the level of plasma urea.

Conclusion: Among the three doses, 80 mg/kg b.w. of naringin exhibited a more efficient antihyperammonemic effect in NH4Cl-induced hyperammonemic rats. Therefore, this study proves the protective effect of naringin in NH4Cl-induced hyperammonemic rats in an apparent dose-dependent manner.

Keywords: ammonia; creatinine; hyperammonemia; naringin; urea

1. Introduction

Ammonia is a neurotoxin that is implicated in the pathogenesis of several metabolic encephalopathies. Ammonia concentration is increased as a result of hepatic dysfunction or encephalopathy through a variety of impaired mechanisms. In hyperammonemic conditions, ammonia diffuses from the blood into the brain and produces neurotoxic effects. Antiepileptic drugs such as valproate and salicylate cause hyperammonemia in mammalian systems. The most severe effects of high ammonia levels occur in the brain characterized by personality and behavior changes, confusion, and tremors, which may even lead to coma and death.

Screening and appraisal of drugs, essentially from traditional medicinal plants and natural products, for their antihyperammonemic activity are currently being pursued. This can be achieved by focusing research on active principles (phytochemicals). Activities of flavonoids are related to their chemical structures, mainly the position and degree of the hydroxyl group, which is important to exert their biological and pharmacological properties.

Flavonoids are naturally occurring polyphenolic compounds that are thought to have positive effects on human health. Naringin (4',5,7-trihydroxy flavone 7-rhamnoglucoside; Figure 1) is one of the flavonoid compounds which is abundantly found in grapefruit and citrus-related species. In recent years, a great deal of attention has been focused on the role of flavonoids in the prevention of chronic diseases. Several studies have reported that naringin
has several biological effects, such as antiulcer, renoprotective, cardioprotective, anticancer, antimitogenic, and anti-inflammatory effects. Furthermore, it was found that naringin was able to traverse the blood–brain barrier. Naringin has empirically been proved to have no side effects, as humans have been ingesting grapes and citrus fruits for a long time.

Accordingly, this study was designed to determine the possible protective effect of naringin on the levels of blood ammonia, plasma urea, uric acid, serum creatinine, and bilirubin and on body weight changes in ammonium chloride (NH₄Cl)-induced hyperammonemia in albino Wistar rats.

2. Materials and methods

2.1. Experimental animals

This study was carried out in male albino Wistar rats (180–200 g) obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital (Reg. No. 160/1999/ CPCSEA; Approval No. 1076, 17.04.2014), Annamalai University, Tamil Nadu, India. The rats were housed in polypropylene cages (47 cm × 34 cm × 20 cm) lined with husk, renewed every 24 hours under a 12:12-hour light/dark cycle at 23 ± 2°C, and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Hyderabad, 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 was approved by the Animal Ethical Committee of Annamalai University, in accordance with the guidelines of National Institute of Nutrition, Hyderabad, India.

2.2. Chemicals

Naringin was purchased from Sigma Chemical Company (St Louis, MO, USA). NH₄Cl and other chemicals used in this study were of analytical grade and obtained from E. Merck-Bangalore and HIMEDIA-Mumbai (India).

2.3. Experimental induction of hyperammonemia

Hyperammonemia was induced in Wistar rats by intraperitoneal injections of a freshly prepared solution of NH₄Cl at a dose of 100 mg/kg body weight (b.w.) 3 times/wk for 8 consecutive weeks.

2.4. Experimental design

The animals were randomized and divided into six groups of six animals each, as shown in the following:

- Group 1: normal rats
- Group 2: rats orally administered with naringin (160 mg/kg b.w.)
- Group 3: rats treated with NH₄Cl (100 mg/kg b.w.) intraperitoneally
- Group 4: rats treated with NH₄Cl + naringin (40 mg/kg b.w.)
- Group 5: rats treated with NH₄Cl + naringin (80 mg/kg b.w.)
- Group 6: rats treated with NH₄Cl + naringin (160 mg/kg b.w.)

At the end of the experimental period (8th week), all rats were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected for the estimation of blood ammonia, plasma urea, uric acid, serum creatinine, and bilirubin.

2.5. Measurement of body weight

During the experimental period, body weights of experimental rats were measured every week using a digital balance.

2.6. Biochemical estimations

2.6.1. Determination of blood ammonia

To determine the levels of ammonia in the blood, triethanolamine (151mM, 200 μL) buffered substrate (α-ketoglutarate) was added to the blood (20 μL), mixed thoroughly, and the absorbance was measured at 480 nm.

2.6.2. Estimation of plasma urea

The level of plasma urea was estimated by adding 0.2 mL of plasma, and 3.5 mL of double-distilled water was added to then. Then 0.35M sulfuric acid (0.4 mL) and 10% sodium tungstate (0.3 mL) were added and centrifuged. To the supernatant (2.1 mL), diacetyl monoxime (0.5 mL), water (2 mL), and sulfuric acid–phosphoric acid mixture (1.6 mL) were added, the mixture was boiled for 30 minutes and cooled, and the absorbance was read at 480 nm.

2.6.3. Estimation of plasma uric acid

Plasma uric acid was determined by adding tungstic acid (5.4 mL) to plasma (0.6 mL). The contents were centrifuged.
To 3 mL of supernatant, 20% sodium carbonate (0.6 mL) and 1% phosphotungstic acid reagent (0.6 mL) were added, the mixture was incubated at 25°C for 10 minutes, and the absorbance was read at 700 nm.27

2.6.4. Estimation of serum creatinine

Serum creatinine was determined by adding alkaline picrate (2 mL) to 3 mL of deproteinized supernatant (prepared by centrifugation of 0.2 mL of serum with 4.3 mL of 10% tricarboxylic acid cycle). The mixture was kept at 25°C for 30 minutes and the absorbance was read at 520 nm.28

2.6.5. Estimation of serum bilirubin

The level of bilirubin in serum was determined by adding absolute methanol (2.5 mL) 1.5% hydrochloric acid (0.5 mL), and a diazo reagent (0.5 mL) to serum (0.2 mL), which were mixed thoroughly and kept at room temperature for 30 minutes, and the absorbance was measured at 540 nm.29

2.7. Statistical analysis

The statistical significance was expressed by one-way analysis of variance using the Software Package for the Social Science (SPSS) Version 15, followed by Duncan’s multiple range test. Each group contained six animals and the results were expressed as mean ± standard deviation. A p value <0.05 was considered statistically significant.30

3. Results

Figure 2 shows a significant decrease in the final body weight of NH4Cl-treated rats as compared with normal rats. No significant change was observed in rats treated with naringin alone compared with rats. Naringin-treated hyperammonemic rats showed nearly normalized body weights as compared with hyperammonemic rats.

NH4Cl-induced hyperammonemic rats showed a significant increase in the levels of blood ammonia and plasma uric acid, and a decrease in the level of plasma urea compared with the normal rats. Oral administration of naringin (40 mg/kg, 80 mg/kg, and 160 mg/kg b.w.) for a period of 8 consecutive weeks to hyperammonemic rats was found to significantly decrease the levels of blood ammonia and plasma uric acid, and increase the level of plasma urea. Among the three doses, the maximum lowering effect of naringin was observed at the dose of 80 mg/kg b.w. (Figures 3–5).

Figures 6 and 7 demonstrate the levels of serum creatinine and bilirubin in experimental rats. A significant increase in the levels of serum creatinine and bilirubin was observed in hyperammonemic rats. Oral administration of naringin to hyperammonemic rats had significant changes in these levels to near normal. Naringin showed a highly significant effect at a dose of 80 mg/kg b.w. compared with the effects at doses of 40 mg/kg and 160 mg/kg b.w. Based on these data, the effective dose was fixed at 80 mg/kg b.w. Hence, further studies were carried out using this effective dose.

4. Discussion

Ammonia is a key factor in the pathogenesis of hepatic encephalopathy, a major complication in acute and chronic liver failure and other hyperammonemic states, such as inborn errors of urea synthesis, during hepatic inadequacy, large quantities of ammonia in portal blood escapes, from the detoxification process and enters systemic circulation. Thus, blood and tissue (brain) ammonia levels are elevated rapidly.31 Ammonia is a potent inhibitor of α-ketoglutarate dehydrogenase, the rate limiting enzyme in tricarboxylic acid cycle causing the accumulation of α-ketoglutarate, which stimulates glutamate formation in both astrocytes and neurons.32 Previous studies33 have reported that hyperammonemic rats showed an increased body weight due to the accumulation of lipid metabolites, proteins, and amino acids in tissues and blood circulation. In our study, naringin-treated hyperammonemic rats showed near normalized body mass as compared with...
hyperammonemic rats. Our results suggest that naringin supplementation (at a dose of 80 mg/kg b.w.) to hyperammonemic rats has managed to have an affirmative effect in opposition to hyperlipidemia.

The liver contains all the detoxification enzymes that are needed for the urea cycle, which helps in the excretion of excess ammonia. The increased blood ammonia and decreased urea indicate the hyperammonemic condition in NH₄Cl-treated rats. This may be due to the liver damage caused by the urea cycle disorder and ammonia intoxication. Naringin-administered hyperammonemic rats showed a significantly decreased level of circulatory ammonia and an increase in urea biosynthesis when compared with corresponding NH₄Cl-treated rats. Various investigations have documented that phytochemicals containing flavonoids offer ammonia detoxification by removing excess ammonia, uric acid, and creatinine during various disease conditions such as hyperammonemia, nephrotoxicity, etc. The reduction in ammonia and enhancement in urea synthesis showed anti-hyperammonemic effects of naringin, favoring normalization of urea cycle defects and reduction of hyperammonemic complications. These observations clearly indicate that naringin could exert a potent antihyperammonemic effect.

Administration of NH₄Cl to rats exhibited a significant increase in plasma uric acid and serum creatinine, and a decrease in plasma urea concentration when compared with the control group. Blood urea nitrogen, uric acid, and creatinine levels are useful indicators of renal function. Renal damage can be accompanied by an increase in blood urea nitrogen, uric acid, and creatinine, indicating reduced urea, uric acid, and creatinine clearance. In addition to the hepatic damage, renal damages were also present, as was evident by the elevation in plasma urea levels, which was considered as a significant marker of renal dysfunction. The earliest research investigated that the levels of plasma uric acid and serum creatinine were increased, and the level of plasma urea concentration was decreased after the administration of NH₄Cl. It might be due to dysfunctional and dystrophic changes in the liver and kidney due to severe renal impairments; as a result, urea excretion decreased and its concentration in plasma increased rapidly. Hyperammonemic rats treated with naringin showed significantly decreased levels of plasma uric acid and serum creatinine, and an increase in plasma urea concentration when compared with NH₄Cl-
treated rats, indicating the antihyperammonemic effect of naringin.

Serum bilirubin is used as an index for the assessment of hepatic function, and any abnormal increase in the level of bilirubin in the serum indicates hepatobiliary diseases and severe disturbance of the hepatocellular function. In the present investigation, the rats induced with NH4Cl showed a significantly increased level of bilirubin as compared with untreated normal rats.

5. Conclusion

Naringin is abundantly present in grapefruit. The juice composition is very different from that of sweet orange, but quite similar to that of grapefruit, being rich in naringin (24, 1.96 mg/100 mL). Therefore, approximately 2 L of grape juice is needed for 40 mg/kg b.w. of naringin that one adult needs to consume daily. Therefore, grape juice is quite similar to that of grapefruit, being rich in naringin (24, 1.96 mg/100 mL). Therefore, approximately 2 L of grape juice is needed for 40 mg/kg b.w. of naringin that one adult needs to consume daily.

Conflict of interest

No conflict of interest.

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