Research Article

Protective Role of *Monolluma quadrangula* Extract Against Hyperammonemia, Oxidative Stress and Inflammation in Ammonium Chloride-induced Rats

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Abstract

**Background and Objectives:** Excess ammonia results from liver failure can cause serious neuropsychiatric complications. *Monolluma quadrangula* is a succulent plant with promising antioxidant and anti-inflammatory properties; however, its protective effect against hyperammonemia hasn’t been reported. The present study was aimed to evaluate the effect of *M. quadrangula* extract on ammonia levels, oxidative stress, inflammation and Na⁺/K⁺-ATPase in ammonium chloride (NH₄Cl)-administered rats. **Materials and Methods:** Rats received NH₄Cl three times per week and 250 mg kg⁻¹ b.wt., *M. quadrangula* extract for 8 weeks. The animals were sacrificed and blood, cerebrum, liver and kidney samples were collected for analyses. **Results:** The NH₄Cl-administered rats showed a significant increase in blood ammonia and liver and kidney function markers. Lipid peroxidation was increased, superoxide dismutase and catalase were decreased in the brain, liver and kidney of rats. Treatment with *M. quadrangula* extract ameliorated blood ammonia, liver and kidney function markers, lipid peroxidation, superoxide dismutase and catalase in NH₄Cl-induced rats. *Monolluma quadrangula* extract prevented inflammation and decreased nitric oxide and glutamine levels in the brain of rats. In addition, *M. quadrangula* extract decreased the expression and activity of Na⁺/K⁺-ATPase in the brain of rats received NH₄Cl. **Conclusion:** These results showed the protective effect of *M. quadrangula* extract against NH₄Cl-induced hyperammonemia. *Monolluma quadrangula* prevented oxidative stress and inflammation and modulated glutamate-nitric oxide-cGMP pathway and Na⁺/K⁺-ATPase in the brain of rats.

**Key words:** Neuropsychiatric complications, excess ammonia, oxidative stress, *Monolluma quadrangula*, succulent plant, hyperammonemia

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**Data Availability:** All relevant data are within the paper and its supporting information files.
INTRODUCTION

Ammonia is a byproduct of nitrogen metabolism that is detoxified in hepatocytes via the urea cycle. Excess ammonia occurs subsequent to liver failure can cause hepatic encephalopathy (HE) and senescence in astrocytes as well as cognitive impairment. Although the exact mechanism of ammonia-induced HE is not fully understood, several studies have pointed to different factors including oxidative stress, inflammation and others. Excess ammonia increased the levels of nitric oxide via reducing glutamate uptake, activation of N-methyl-D-aspartic acid (NMDA) receptors and increased intracellular calcium (Ca++) levels which activates neuronal nitric oxide synthase (nNOS). In addition, excess ammonia and nitric oxide activate soluble guanylate cyclase and increase the levels of cyclic guanosine monophosphate (cGMP). It was shown that ammonia can induce nucleic acid oxidation in cultured astrocytes and in the brain. Therefore, hepatic encephalopathy manifests as oxidative/nitrosative stress along with low-grade cerebral edema. In this context, studies have demonstrated that excess ammonia is associated with increased lipid peroxidation and nitric oxide in the brain of animals. Inflammation has also been implicated in hepatic encephalopathy and liver failure resulted in increased inflammatory cytokines in the brain and in the circulation.

Given the role of hyperammonemia, oxidative stress and inflammation in the pathogenesis of hepatic encephalopathy, agents with antioxidant and anti-inflammatory effects can provide protection against the deleterious effect of excess ammonia. Monilloma quadrangular (Forssk.) is a plant with irregularly branched and compressed stem. This succulent bush has been applied in folk medicine for treating peptic ulcer and diabetes mellitus. Recently, Bin-Jumah has reported the antioxidant, anti-inflammatory, glucose-lowering and hepatoprotective properties of M. quadrangular extract in hypercholesterolemic and diabetic rats. In these animal models, M. quadrangular decreased lipid peroxidation and increased the levels of antioxidants. In addition, M. quadrangular extract showed antioxidant and protective effects against gastric ulcer in rodents. Furthermore, different extracts of M. quadrangular have exerted anti-hyperglycemic effect in diabetic rats. However, the effect of M. quadrangular on oxidative stress and inflammation in hyperammonemia have not been reported. So the present study was investigated the protective effects of M. quadrangular against excess ammonia, oxidative stress, nitric oxide and inflammation in ammonium chloride (NH₄Cl)-administered rats.

MATERIALS AND METHODS

Preparation of M. quadrangular extract: The collection of M. quadrangular and preparation of its ethanolic extract were conducted as previously reported by Bin-Jumah. In brief, the plant was collected from Abha (Saudi Arabia), dried and powdered using an electric grinder. The powder was soaked in ethanol/water (1:1 vol/vol) for 24 h, filtered and dried using rotary evaporator.

Animals and experimental design: Male Wistar rats weighing 180-200 g obtained from the animal house of King Saud University, were used in the present study. The rats were housed in standard cages at normal atmospheric temperature and 12 h dark/light cycle and were supplied a standard pellet diet and water ad libitum. All animal procedures were approved by the Institutional Animal Ethics Committee of Jof University (Approval number, 219/34).

Twenty-four rats were divided into four groups (N = 6) as following:

- **Group I (Control)**: Rats received normal saline (3 times/week) and 0.5% carboxymethyl cellulose (CMC) daily for 8 weeks
- **Group II (M. quadrangular)**: Rats received saline (3 times/week) and 250 mg kg⁻¹ b.wt., M. quadrangular extract dissolved in 0.5% CMC daily for 8 weeks
- **Group III (NH₄Cl)**: Rats received 100 mg kg⁻¹ NH₄Cl (Sigma, USA) dissolved in saline (3 times/week; ip.) and 0.5% CMC daily for 8 weeks
- **Group IV (NH₄Cl+M. quadrangular)**: Rats received NH₄Cl (3 times/week, ip.) and 250 mg kg⁻¹ b.wt., M. quadrangular extract daily for 8 weeks

Both saline and NH₄Cl were administered intraperitoneally, whereas, CMC and M. quadrangular extract were administered via oral gavage.

At the end of the experiment, overnight fasted rats were sacrificed and blood, brain, liver and kidney were collected for analysis. The brain was dissected and cerebrum was isolated. The cerebrum, liver and kidney were homogenized in cold phosphate buffered saline (10% w/v) and the homogenates were centrifuged and the clear supernatant collected for the analysis of lipid peroxidation, superoxide dismutase (SOD) and catalase. In addition, nitric oxide, Na⁺/K⁺-ATPase, glutamine, tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) were assayed in the cerebral homogenate. Other samples from the cerebrum were kept frozen for the gene expression analysis.
ASSAY OF BLOOD AMMONIA, LIVER AND KIDNEY FUNCTION MARKERS: Ammonia was assayed in whole blood samples using a reagent kit purchased from Spinreact (Spain)\(^1\), where as alanine aminotransferase (ALT)\(^2\), aspartate aminotransferase (AST)\(^3\), alkaline phosphatase (ALP)\(^4\) and creatinine\(^5\) were determined in serum using Spinreact (Spain) reagent kits.

ASSAY OF LIPID PeroXIDATION, SOD AND CATALASE: Lipid peroxidation, SOD and catalase were assayed in the cerebrum, liver and kidney of rats according to the methods of Preuss et al\(^6\), Marklund and Marklund\(^7\) and Cohen et al\(^8\), respectively.

ASSAY OF NITRIC OXIDE, GLUTAMINE, TNF-\(\alpha\) AND IL-1\(\beta\): Nitric oxide and glutamine levels were measured in the cerebrum of rats following the method of Grisham et al\(^9\) and Lund\(^10\), respectively. The TNF-\(\alpha\) and IL-1\(\beta\) were determined using ELISA kits (R and D Systems, USA), according to the manufacturer’s instructions.

ASSAY OF CEREBRAL Na\(^+\)/K\(^+\)-ATPase ACTIVITY: The activity of Na\(^+\)/K\(^+\)-ATPase was determined in the cerebrum of rats through the assessment of the levels of inorganic phosphate (Pi) liberated from ATP\(^11\). The liberated Pi was determined using Spinreact kits\(^12\).

ASSAY OF Na\(^+\)/K\(^+\)-ATPase GENE EXPRESSION: Total RNA, isolated from the cerebrum samples using Trizol (Invitrogen, USA) was treated with DNase I and quantified at 260 nm using a nanodrop. The RNA with A260/280 nm ratio of 1.8 or more was selected and 2 \(\mu\)g was reverse transcribed into cDNA using RT kit (Thermo Scientific, USA). The cDNA was then amplified by SYBR green master mix (Fermentas, USA)\(^13\) and the following primers: Na\(^+\)/K\(^+\)-ATPase, F: TGGCATCCGAA GTGCTACAG and R: CCAGATCACAACGAGCACA; and \(\beta\)-actin, F: CCGCGATCAACCTCTTG and R: CAGTTGCTGACAA TGCCCGTG. The amplification data were analyzed using the 2\(^{-}\Delta\Delta CT\) method\(^14\).

STATISTICAL ANALYSIS: Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA) and all results were expressed as mean \(\pm\) standard error of the mean (SEM). The statistical comparisons were made by means of the one-way ANOVA test followed by Tukey’s test post hoc analysis. A p<0.05 was considered significant.

RESULTS

Effect of *M. quadrangula* on blood ammonia levels: Oral administration of *M. quadrangula* didn’t cause significant changes in blood ammonia of control rats as shown in Fig. 1. NH\(_4\)Cl-administered rats showed significant increase in blood ammonia (p<0.001). The NH\(_4\)Cl-administered rats treated with *M. quadrangula* showed reduced blood ammonia (p<0.001, Fig. 1).

Effect of *M. quadrangula* on liver and kidney function:

Rats administered with NH\(_4\)Cl showed liver injury evidenced by the significant increase in serum ALT (Fig. 2a), AST (Fig. 2b) and ALP (Fig. 2c) as compared to the control rats. Kidney function has also been altered in NH\(_4\)Cl-administered rats as shown by increased serum creatinine (p<0.001, Fig. 2d). On the other hand, NH\(_4\)Cl-administered rats treated with *M. quadrangula* showed improved (p<0.001) liver and kidney function, whereas, control rats received *M. quadrangula* didn’t show changes in liver and kidney function (Fig. 2d).

*Monlloma quadrangula* ameliorates lipid peroxidation and enhances antioxidants:

The brain of rats received NH\(_4\)Cl showed a significant increase (p<0.001) in lipid peroxidation as compared to the control group (Fig. 3a). On the other hand, the activity of SOD (Fig. 3b) and catalase (Fig. 3c) was decreased significantly (p<0.01) in the brain of NH\(_4\)Cl-administered rats. Treatment with *M. quadrangula* extract decreased lipid peroxidation (p<0.001) and increased SOD (p<0.001) and catalase (p<0.01) in the brain of the NH\(_4\)Cl-administered rats (Fig. 3c).

![Fig. 1: Effect of *M. quadrangula* ameliorates blood on ammonia levels in NH\(_4\)Cl-administered rats](image)
Similarly, lipid peroxidation was significantly increased in the liver of rats received NH₄Cl (p<0.001) as represented in Fig. 4a. Hepatic SOD (Fig. 4b) and catalase (Fig. 4c) were decreased significantly (p<0.01) in NH₄Cl-administered rats. The supplementation of *M. quadrangular* decreased lipid peroxidation and increased SOD and catalase in the liver of NH₄Cl-administered rats, whereas, no effect has showed in the control rats.

The NH₄Cl administration increased lipid peroxidation (Fig. 5a), decreased the activity of SOD (Fig. 5b) and catalase (Fig. 5c) significantly (p<0.001) in the kidney of rats when compared with the control group (Fig. 5c). Oral supplementation of *M. quadrangular* extract decreased kidney lipid peroxidation and increased SOD and catalase in NH₄Cl-administered rats, whereas, no effect has showed in the control rats (Fig. 5c).

**Monolluma quadrangular attenuates inflammation in the brain of rats:** The NH₄Cl-administered rats showed a significant (p<0.001) increase in cerebral TNF-α (Fig. 6a) and IL-1β (Fig. 6b) as compared to the control group. The NH₄Cl-administered rats treated with *M. quadrangular* extract showed a significant (p<0.001) decrease in the cerebral levels of TNF-α and IL-1β. Oral supplementation of *M. quadrangular* extract didn’t cause significant changes in pro-inflammatory cytokines in the cerebrum of control rats.

**Monolluma quadrangular on alleviates nitric oxide, glutamine and Na⁺/K⁺ ATPase activity in the brain of rats:**

The NH₄Cl-induced hyperammonemia resulted in a significant increase in cerebral nitric oxide (p<0.01, Fig. 7a) and glutamine levels (p<0.001, Fig. 7b) as compared to the control rats. NH₄Cl-administered rats treated with *M. quadrangular* showed a significant decrease in cerebral nitric oxide (p<0.01) and glutamine levels (p<0.001). On the other hand, rats received *M. quadrangular* extract showed non-significant changes in cerebral nitric oxide and glutamine levels.

The NH₄Cl-administered rats showed a significant (p<0.001) increase in the activity (Fig. 7c) as well as the gene expression (Fig. 7d) of cerebral Na⁺/K⁺ ATPase. Treatment of the NH₄Cl-administered rats with *M. quadrangular* extract significantly (p<0.001) ameliorated the expression and activity of cerebral Na⁺/K⁺ ATPase. *Monolluma quadrangular* extract caused non-significant changes in the expression and activity of cerebral Na⁺/K⁺ ATPase in normal rats (Fig. 7c, d).
**DISCUSSION**

*Monontuma quadrangula* extract has been recently reported to protect against oxidative stress and inflammation in hypercholesterolemic and diabetic rats\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\). The present study showed that the *M. quadrangula* extract prevents oxidative damage and inflammation and modulates glutamate-NO-cGMP signaling in the cerebrum of NH\(_4\)Cl-administered rats.

The NH\(_4\)Cl-induced rats in the present investigation showed a significant increase in blood ammonia levels, liver and kidney injury manifested by the increased levels of serum ALT, AST, ALP and creatinine. The administration of NH\(_4\)Cl for 8 weeks has been demonstrated to increase the blood ammonia levels as a consequence of liver injury\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\). It was studied that the hyperammonemia is a direct consequence of liver injury\(^7\) and hepatoprotective effects can prevent hyperammonemia and its associated deleterious effects. In the present study, treatment of the NH\(_4\)Cl-administered rats with *M. quadrangula* extract prevented both liver and kidney injury as showed by the ameliorated levels of ALT, AST, ALP and creatinine. In addition, *M. quadrangula* extract prevented NH\(_4\)Cl-induced hyperammonemia in rats which is a direct result of its hepatoprotective and renoprotective effects.
Fig. 5(a-c): Effect of *M. quadrangular* on (a) Lipid peroxidation, (b) SOD and (c) Catalase in the kidney of NH4Cl-administered rats
Data are expressed as Mean±SEM (N = 6). ***p<0.001 vs. control and ###p<0.001 vs. NH4Cl

Previous studies have demonstrated that oxidative stress is implicated in the deleterious effects of excess ammonia. The brain is vulnerable to damage by oxidative stress because of its low content of antioxidant and high content of polyunsaturated fatty acids. In the present study, NH4Cl-administered rats showed oxidative stress in the brain, liver and kidney evidenced by the increased levels of lipid peroxidation, declined antioxidant enzymes SOD and catalase. In agreement with these findings, recent work from Mahmoud’s lab showed increased lipid peroxidation and decreased antioxidant enzymes in the brain, liver and kidney of hyperammonemic rats. Interestingly, treatment of the NH4Cl-administered rats with *M. quadrangulara* extract prevented lipid peroxidation and alleviated SOD and catalase in the brain, liver and kidney. Therefore, the antioxidant efficacy of *M. quadrangulara* extract seems to play an important role in its protective effect against hyperammonemia. In agreement with the observed antioxidant effects of *M. quadrangulara* extract, Bin-Jmah has recently reported decreased in oxidative stress and increased in levels of enzymatic and non-enzymatic antioxidants in the liver of hypercholesterolemic and diabetic rats. In addition, *M. quadrangulara* extract prevented lipid peroxidation in the stomach of a rat model of peptic ulcer. The rich content of glycosides and phenolics have been reported to be the main contributor of the antioxidant effect of *M. quadrangulara* extract.

In addition to mitigating oxidative stress, *M. quadrangulara* extract showed a potent anti-inflammatory activity in the brain of NH4Cl-administered rats. Treatment of the rats with *M. quadrangulara* extract alleviated the levels of TNF-α and IL-1β in the brain of hyperammonemic rats. In hyperammonemia, inflammation has been associated with neuropsychological alterations and studies have shown the role of inflammation in the pathogenesis of hepatic encephalopathy. Pro-inflammatory cytokines have been
Fig. 7(a-d): Effect of *M. quadrangula* (a) Nitric oxide, (b) Glutamine, (c) Na⁺/K⁺-ATPase activity and (d) Na⁺/K⁺-ATPase gene expression in the cerebrum of NH₄Cl-administered rats

Data are expressed as Mean±SEM (N = 6). **p<0.01, ***p<0.001 vs. control and "p<0.01, **p<0.001 vs. NH₄Cl

*Fig. 7 (a-d):* Effect of *M. quadrangula* (a) Nitric oxide, (b) Glutamine, (c) Na⁺/K⁺-ATPase activity and (d) Na⁺/K⁺-ATPase gene expression in the cerebrum of NH₄Cl-administered rats. 

Data are expressed as Mean±SEM (N = 6). **p<0.01, ***p<0.001 vs. control and "p<0.01, **p<0.001 vs. NH₄Cl.

**Discussion:**

Nitric oxide demonstrated to increase in NH₄Cl-administered rats and in patients with hepatic cirrhosis. Due to the implication of inflammation in excess ammonia-induced neuropsychological alterations, agents with anti-inflammatory effects can prevent these alterations. In agreement with current results, recent studies showed the anti-inflammatory effect of *M. quadrangula* extract in hypercholesterolemic and diabetic rats.

To further explore the protective mechanism of *M. quadrangula* extract against hyperammonemia, the cerebral levels of nitric oxide, glutamine, activity and expression of Na⁺/K⁺ ATPase were determined. Nitric oxide was increased in the brain of rats received NH₄Cl which is in agreement with previous reports. The increased nitric oxide is a direct result of increased in nNOS expression. Excess ammonia activates NMDA glutamate receptors resulting in opening of the Ca²⁺ channels, activation of nNOS and increased production of nitric oxide which increases the formation of cGMP. Previously, the increased expression of nNOS in the cerebrum of rats was reported by different researchers. In addition to the increased nitric oxide, NH₄Cl-administered rats showed the increased levels of the amino acid glutamine which is a result of the condensation of glutamate and ammonia. The synthesis of glutamine by glutamine synthetase in astrocytosis increased in hyperammonemia and excess levels of glutamine can alter the cerebral blood flow and cause oxidative stress, osmotic imbalance and cerebral edema. Previous studies have demonstrated the increased glutamine levels in the cerebrum of rats received NH₄Cl. Treatment of the NH₄Cl-administered rats with *M. quadrangula* extract reduced the cerebral levels of nitric oxide and glutamine which could be attributed to the decreased ammonia levels. Therefore, the modulatory effect of *M. quadrangula* extract on the ammonia-induced glutamate-nitric oxide-cGMP pathway plays a role in its protective effect against hyperammonemia.

Furthermore, *M. quadrangula* extract ameliorated the expression and activity of Na⁺/K⁺-ATPase in the cerebrum of NH₄Cl-administered rats. Several studies showed previously that the activity of Na⁺/K⁺-ATPase increases in the brain of hyperammonemic animals. Along with the *in vivo* studies, *in vitro* experiments showed increased Na⁺/K⁺-ATPase activity in murine astrocytes exposed to ammonia. The neurotoxic effect of ammonia is associated with compromise K⁺ buffering in astrocytes. Therefore, *M. quadrangula* extract maintained K⁺ buffering through modulating Na⁺/K⁺-ATPase and prevent the deleterious effects of excess ammonia on the astrocytes.
CONCLUSION

The results of this study showed for the first time that *M. quadrangula* extract protected against excess ammonia in rats. *Monolaura quadrangula* prevented oxidative stress, inflammation and decreased the glutamate-nitric oxide-cGMP pathway and activity of the Na⁺/K⁺-ATPase. Hence, *M. quadrangula* can provide protection against hyperammonemia and its associated neuronal alterations.

SIGNIFICANCE STATEMENT

Treatment of the hyperammonemic rats with *Monolaura quadrangula* ameliorated blood ammonia levels and prevented ammonium chloride-induced liver and kidney dysfunction. This study shows for the first time that *Monolaura quadrangula* protects against excess ammonia associated oxidative stress in the brain, liver and kidney of rats. In addition, the results show that modulation of inflammation, glutamate-nitric oxide-cGMP pathway and Na⁺/K⁺-ATPase activity in the brain is the main mechanism of the protective effect of *Monolaura quadrangula* against hyperammonemia.

REFERENCES


