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Research Article

Effect of *musa paradisiaca l.* extract against hyperammonemia in rats

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ABSTRACT

This investigation was designed to determine the possible protective effect of root stock of *Musa paradisiaca l* extract (MP) against ammonium chloride (NH4Cl) induced hyperammonemia. Experimental hyperammonemia was induced in adult male Wistar rats (180–200 g) by intraperitoneal injections of NH4Cl (100 mg/kg body weight) thrice a week. Rats were treated with MP (300 mg/kg body weight) via oral administration. At the end of experimental duration, blood ammonia, plasma urea, serum liver marker enzymes and lipid peroxidation in plasma and tissues (liver and brain) of normal and experimental animals were analysed. The results revealed that NH4Cl induced hyperammonemia, as evidenced by increases in the levels of blood ammonia, plasma urea, lipid peroxidations such as thiobarbituric acid reactive substances (TBARS), hydroperoxides (HP) and activities of liver markers (alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) and the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase and reduced glutathione (GSH), were decreased in liver and brain tissues of NH4Cl group compared with the normal group. On the other hand, MP treatment reversed all these biochemical indices. The study shows wider evidence for the antihyperammonemic, hepatoprotective, neuroprotective and antioxidant activities of MP extract against NH4Cl induced hyperammonemia**.**

Keywords: *Musa paradisiaca;* hyperammonemia; Lipid peroxidation; Antioxidants; Liver marker enzymes.

INTRODUCTION

In mammals, ammonia is an important nitrogen substrate in several reactions, and plays an important role in nitrogen homeostasis of mammalian cells. Ammonia is produced by amino acid and protein catabolism and is toxic to brain and muscles. Ammonia toxicity results in free-radical generation that leads to oxidative stress and tissue damage (Lena et al, 1996). Hyperammonemia may result from a genetic defect or acquired conditions such as Reye's syndrome, high-dose chemotherapy and severe infection (Treem et al, 1994), it is a major contributing factor to neurological abnormalities observed in hepatic encephalopathy and in congenital defects of ammonia detoxication (Monfort et al, 2005). Ammonia is a neurotoxic and enters the brain via the blood brain barrier, which might result in central nervous system symptoms of dysfunction such as energy metabolism (Rao et al, 2001), hemodynamics (Vaquero et al, 2004), neurotransmission (Felipo etal, 2002) ataxia, mental confusion, syncope and in severe cases to coma and death (Ott et al, 2007). The screening and development of drugs for their antihyperammonemic activity is still in progress, and there is thus a worldwide trend to investigate traditional medicinal plants and natural products, there is a need to search for appropriate protective agents against hyperammonemia. Accordingly, this study was designed to determine the possible protective effect of MP on blood ammonia, plasma urea, liver marker enzymes and tissues (brain and liver) lipid peroxidation and antioxidant status in NH4Cl induced hyperammonemia in rats.

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MATERIALS AND METHODS

Animals: Adult male albino Wistar rats, weighing 180–200 g obtained from National institute of Biosciences, Pune. The animals were housed in polycarbonate cages in a room with a 12 h day–night cycle, temperature of 22 ± 2°C and humidity of 45– 64%. Animals were fed with a standard pellet diet and water ad libitum. All animal experiments were approved by the Intuitional Animal Ethical committee, JSPM'S College of Pharmacy & Research, Wagholi, Pune.

Collection and identification of plant

The root stocks of the plant was collected from agriculture fields of Pune and identified by botanical survey of India (BSI) Pune; voucher specimen no NAVMUP2 deposited in JSPM'S Charak College of pharmacy and research Wagholi, Pune.

Preparation of the extract

The dried root stocks were grounded to get coarse powder. The ethanolic extract of root stock was pre-

Table 1: Effect of MP on changes in the blood ammonia and plasma urea, serum AST, ALT and ALP, TBARS and HP in plasma, liver and brain of normal and experimental rats

Biochemical parameters	Normal	Normal +MP	NH4Cl (100 mg/kg)	MP (300 Mg/kg) + NH4Cl
Blood ammonia (mol/L)	85.16±2.03a	84.16±5.68a	323.16±8.91b	145.0±6.91c
Urea (mg/dl)	12.58±0.78a	12.08±0.53a	24.83±0.55b	16.16±0.68c
AST (IU/L)	75.48±2.68a	73.11±3.62a	105.92 ±5.62b	84.58±3.76c
ALT (IU/L)	24.58±2.32a	24.58±2.32a	49.48±2.0b	32.21±0.87c
ALP (IU/L)	71.75±6.73a	73.32±4.82a	134.83±7.50b	84.44±5.05c
Plasma TBARS (nM/ml)	2.95±0.16a	2.85±0.28a	4.6±0.37b	3.4±0.22c
Plasma HP (values x 10-5 mM/dl)	8.15±0.75a	7.91±0.69a	14.15±0.93b	10.47±0.89c
Liver TBARS (mM/100g wet tissue)	0.80±0.04a	0.82±0.51a	2.1±0.22b	1.35±0.18c
Liver HP (mM/100g	65.47±2.66a	66.06±3.04a	96.42±3.57b	78.56±4.12c
Brain TBARS (mM/100g wet tissue)	1.05±0.15a	1.0±0.14a	2.1±0.24b	1.4±0.14c
Brain HP (mM/100 g wet tissue)	109.61±5.25a	108.41±4.87a	132.13±5.56b	122.61±6.41c

Each value is mean ± SD for six rats in each group. Values not sharing a common superscripts (a, b and c) differ significantly at P < 0.05 (DMRT).

Table 2: Effect of MP on the activities of SOD, catalase, GSH and GPx in the liver and brain of normal and experimental rats

Biochemical parameters	Normal	Normal + MP	NH4Cl (100 mg/kg)	MP (300 mg/kg) + NH4Cl
Liver SOD (Ua/mg protein)	9.05 ± 0.42a	9.64 ± 0.67a	3.44 ± 0.32b	6.39 ± 0.43
Liver Catalase (Ub/mg protein)	85.10 ± 5.32a	85.06 ± 6.32a	39.88 ± 4.28b	64.26 ± 5.14c
Liver GSH (mg/100g wet tissue)	51.55±3.97 a	52.33±2.16a	24.88±1.03b	44.43±3.10c
Liver GPx (Uc/mg protein)	9.29 ± 0.50a	9.65 ± 0.24a	4.76 ± 0.83b	7.42 ± 0.34
Brain SOD (Ua/mg protein)	7.23 ± 0.39a	7.12 ± 0.47a	5.14 ± 0.21b	6.23 ± 0.35c
Brain Catalase (Ub/mg protein)	3.12 ± 0.20a	3.20 ± 0.23a	0.86 ± 0.05b	2.73 ± 0.17c
Brain GSH (mg/100g wet tissue)	34.88±2.28a	34.67±2.10a	17.10±1.42b	26.89±2.0
Brain GPx (Uc/mg protein)	3.72±0.41a	3.55 ± 0.34a	1.63 ± 0.17b	2.76 ± 0.16c

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscripts (a, b and c) differ significantly at P < 0.05 (DMRT). Ua–Ua is defined as the enzyme concentration required inhibiting the OD at 560 nm of chromogen production by 50% in 1 min. Ub–Ub l moles of H2O2 consumed/min.

pared using 70% (v/v) alcohol by soxhlet method at temperature of 60-70°C. The extracts were then filtered, concentrated under vacuum dried (Bahuguna et al, 2009).

Experimental design: In the experiment, a total of 24 rats were used. The rats (180– 200g) were divided into 4 groups of 6 rats each. Among them group I: normal rats administered with saline solution 0. 5 ml each; group II: with MP (300mg/kg) administered orally (Ramprasath et al, 2005); group III: NH4Cl (100 mg/kg b. w) intraperitoneally (Subash et al, 2008); group IV: NH4Cl + MP300 (mg/kg). At the end of 8th week, the rats were fasted overnight and sacrificed by cervical dislocation after anaesthetizing the rats with ketamine hydrochloride (30 mg/kg b. wt; im). Blood samples and tissue homogenates were prepared for the estimation of various biochemical parameters.

Biochemical Estimations: Blood ammonia (Wolheim, 1990); Plasma urea (Varley et al 1998); serum AST (Retiman et al, 1957) , ALT (Retiman et al, 1957) and ALP (King et al, 1934) ; TBARS in Plasma (Yagi, 1987), liver and brain (Fraga et al, 1988); HP in plasma and liver and brain (Jiang et al, 1992) ; SOD (Kakkar et al, 1984), catalase (Sinha, 1972), GSH (Ellman et al, 1959) and GPx (Rotruck et al, 1973) in liver and brain, Protein in the enzyme extract (Lowry et al, 1951) were analyzed.

Statistical analysis: Analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9. 05. Results were expressed as mean ± SD from six rats in each group. P values < 0. 05 were considered as significant.

RESULTS

Levels of blood ammonia, plasma urea, serum AST, ALT and ALP; TBARS and HP in plasma, liver and brain tissues were significantly increased in NH4Cl treated rats as compared to normal rats. These levels were significantly decreased in hyperammonemic rats with MP when compared with NH4Cl alone induced rats (Table 1).

Activities of SOD, catalase, GSH and GPx in tissues were found to be significantly decreased in rats induced with NH4Cl when compared with normal rats. These levels were reverted back to near-normal levels in hyperammonemic rats treated with MP when compared with NH4Cl alone induced rats (Table 2).

DISCUSSION

The increased levels of blood ammonia and plasma urea indicate hyperammonemic condition in rats treated with NH4Cl (Ramprasath et al, 2005)which may be due to liver damage caused by ammonia intoxication. Ammonia induced neurotoxicity has been reported the dysfunction of multiple neurotransmitter system including glutamate mediated excitotoxicity, electrophysiological disturbances and defects in brain Bioenergetics (Rao et al, 2005). Reports have shown that excess ammonia induces nitric oxide synthase, which leads to the enhanced production of nitric oxide (NO), leading in turn to oxidative stress and liver damage (Subash et al 2009; Jayakumar et al, 2009). Various investigations have documented that plant extracts containing phonetic compounds and flavonoids offer ammonia detoxication by removing excess ammonia, urea, uric acid and creatinine during various disease conditions such as hyperammonemia, nephrotoxicity, etc (Shirwaikar et al, 2003). Decreased levels of blood ammonia and plasma urea in the MP and NH4Cl treated rats may be due to the antioxidant potential of MP. This study shows the elevated levels of serum liver markers and lipid peroxidation products in tissues of NH4Cl 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-D-aspartate (NMDA) receptors leads to neuronal degeneration and death (Kosenko et al, 1999) due to increased Ca2+ concentration in the postsynaptic neuron (Manev et al, 1989). Ca2+ binds to calmodulin and activates nitric oxide synthase, increasing the formation of NO that contributes to the neurotoxin process (Hermenegildo et al, 2000). Decreased levels of liver markers and lipid peroxidation products in MP administered rats may be due to its free radical scavenging property.

In this study, NH4Cl induced rats exhibited decreased activities of SOD and catalase in liver and brain tissues. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of lipid peroxidation (Jagetia et al, 2003). The decrease in the activities of these antioxidant enzymes might be due to damage of these tissues. MP administration significantly normalized the activities of SOD and catalase in tissues of NH4Cl induced rats. We observed decreased levels of GSH and GPx in tissues of hyperammonemic rats; this might be due to the increased utilization in protecting 'SH'-containing proteins from lipid peroxides. MP treatment significantly increased the levels of GSH and GPx in tissues of NH4Cl induced rats. The present investigation shows that the MP exerts protection to NH4Cl induced hyperammonemia in rats against oxidative stress. This could be due to prevention or inhibition of lipid peroxidation by its antioxidant and hepatoprotective effect.

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