International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue: 7 2014 Pages: 164-168
(pISSN: 2348-5213; eISSN: 2348-5221)

RESEARCH ARTICLE

ANTIOXIDANT EFFECT OF *SEMECARPUS ANACARDIUM*ON AMMONIUM CHLORIDE - INDUCED HYPERAMMONEMIA IN WISTAR RATS

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Abstract

This study was aimed to evaluate the antioxidant effects of *Semecarpus anacardium* nut milk extract (SAE) against ammonium chloride (NH_4Cl) - induced experimental hyperammonemia. Experimental hyperammonemia was induced in adult male Wistar rats (180–200 g) by intra-peritoneal injections of NH_4Cl (100 mg/kg b.wt). Oral administration of SAE (150 mg/kg b.wt) on blood ammonia, plasma urea and antioxidants in kidney tissue of normal and experimental animals were analysed. Administration of SAE in hyperammonemic rats improved the levels superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) in NH_4Cl treated rats. Oxidative stress was effectively modulated by SAE administration. SAE significantly improved the status of antioxidants and decreased ammonia, urea as compared to NH_4Cl treated rats. The study offers evidence for the antihyperammonemic and antioxidant effects of SAE against oxidative stress in the kidney, induced by NH_4Cl.

Keywords: Hyperammonemia, *Semecarpus anacardium,* Catalase, SOD

Introduction

The neurological complications of hyperammonemia in the central nervous system (CNS) are now receiving more attention. Ammonia is a neurotoxin that has been strongly implicated in the pathogenesis of hepatic encephalopathy[1]. Ammonia has also been a major pathogenetic factor associated with inborn errors of urea cycle, Reye's syndrome, organic acidurias, and disorders of fatty acid oxidation[2]. Ammonia-induced neurotoxicity has been reported to include a dysfunction of multiple neurotransmitter system glutamate-mediated excitotoxicity, electrophysiological disturbances, and defects in brain bioenergetics[1,3]. In spite of extensive investigations, the precise mechanisms involved in ammonia neurotoxicity are not completely understood. Oxidative stress is an evolving concept in ammonia neurotoxicity. Its effect on oxidative and nitrosative stress in the CNS has been recently reviewed[1]. Recent studies have reported an increased production of free radicals in cultured astrocytes after treatment with pathophysiological concentrations of ammonia[4]. A concurrent increase of superoxide production and a reduction in the activities of various antioxidant enzymes have been seen in animal models with acute ammonia toxicity[5]. Oxidative stress-mediated lipid peroxidation has also been seen as one of the characteristic features of hyperammonemia[6-8]. Presently available potent synthetic anti-hyperammonemic agents/therapies lie in their toxicity and the reappearance of symptoms after discontinuation[9]. The screening and appraisal of drugs for their anti-hyperammonemic activity is pursuing till date, essentially from traditional medicinal plants and natural products.

Semecarpus anacardium, commonly known as 'marking nut' has high priority and applicability in indigenous system of medicine against various diseases. *Semecarpus anacardium* nut milk extract (SAE)potentiated the efficacy of commonly used anti-cancer drugs like mitomycin, fluorouracil and methotrexate[10]. SAE contains more than 20 active phyto-constituents. SAE was subjected to investigation against hepatocellular carcinoma[11],mammary carcinoma[12] and rheumatoid arthritis[10] in experimental mammals for its beneficial effects. Further, SAE is known to offer hepatoprotective[11], anti-inflammatory[13], antiatherogenic[14], cardio protective[15] and antiglycemic properties[16].

To our knowledge, this report is the first study to investigate the effect of SAE on circulation and kidney lipid peroxidation and antioxidant status in ammonium chloride (NH_4Cl)-induced hyperammonemic rats. Therefore, the objective of the present study is to investigate the influence of SAE on blood ammonia, plasma urea and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) in kidney tissue of an animal model of NH_4Cl -induced hyperammonemia.

Materials and Methods

Experimental animals

All the experiments were carried out in male albino Wistar rats (180–200g), obtained from Central Animal House, Faculty of Medicine, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47×34×20 cm) lined with husk, renewed every 24 h, kept under 12:12 h light/dark cycle at 23±2°C and had free access to drinking water and food. The rats were fed with standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The experiments were 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 AnnamalaiUniversity (Approval no. 537; dated 20/03/2008).

Chemicals

Ammonium chloride was purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals used in the study were of analytical grade.

Semecarpus anacardium nut milk extract (SAE)

The preparation contains purified nuts of *Semecarpus anacardium*, cow's milk and ghee. The SAE was prepared by boiling the nuts (200g) with 500 ml of milk. After decanting the decoction, 500 ml of milk was added to the boiled nuts and the mixture was again boiled for 15 minutes. The decoction was recovered and the process was repeated again with the milk. All the three portions of milk nut decoction were mixed with ghee and boiled till dehydrated, then filtered and stored[17].

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 (b.wt) thrice in a week for 8 consecutive weeks[18,19].

Experimental design

The rats (180– 200 g) were divided into 5 groups of 6 rats each. Group I rats were administered with olive oil (as vehicle) 0.5 ml each; group II rats were administered with SAE (150 mg/kg b.wt. dissolved in 0.5 ml olive oil) orally by using an intragastric tube (thrice a week for 8 consecutive weeks)[10]; group III rats were injected intraperitoneally with NH_4Cl (100 mg/kg b.wt); group IV rats were given NH_4Cl + SAE (thrice a week for 8 consecutive weeks) and SAE control and group V rats (SAE control) were orally administered with milk and ghee extract without SA nuts (thrice a week for 8 consecutive weeks respectively).

At the end of 8th week, the rats were made to fast overnight and sacrificed by cervical dislocation. Blood samples were collected; plasma and serum were separated by centrifugation. Kidney tissue were excised immediately and rinsed in ice-chilled normal saline; 500 mg of the tissues were homogenized in 5.0 ml of 0.1 M Tris-HCl buffer (pH, 7.4). The homogenate was centrifuged and supernatant was used for the estimation of biochemical indices.

Biochemical Estimations

Blood ammonia[20], Plasma urea[21], SOD[22], CAT[23], GPx[24] and GSH[25].

Statistical analysis

Statistical 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 6 rats in each group. *P* values <0.05 were considered as significant.

Results

Table 1 shows the levels of blood ammonia and plasma urea in normal and experimental rats. The levels of circulatory ammonia and urea were significantly higher in NH_4Cl-treated rats when compared with normal. Hyperammonemic rats treated with SAE significantly normalized the levels of ammonia and urea and lipid peroxidation products, as compared with hyperammonemic rats.

Table 2 shows the levels of antioxidants in kidney tissue of normal and experimental groups. The levels of SOD, CAT, GPx and GSH were significantly lower in NH_4Cl-treated rats, and these levels were significantly normalized in hyperammonemic rats treated with SAE.

Discussion

This study is one of the series of studies showing that chronic hyperammonemia causes an imbalance in the oxidative status of the renal tissue and that the resulting free radicals damage the

Table 1: Effect of SAE on changes in the levels of blood ammonia and plasma urea of normal and experimental rats.

Groups	Blood ammonia («mol/L)	Urea (mg/dl)
Normal	85.16±2.03[a]	12.58±0.78[a]
Normal + SAE(150 mg/kg)	84.16±0.68[a]	12.08±0.53[a]
NH_4Cl (100 mg/kg)	323.16±2.91[b]	24.83±0.55[b]
NH_4Cl+ SAE	145.0±1.91[c]	16.16±0.68[c]
SAE control	86.33±1.49[a]	12.41±0.67[a]

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)

Table2: Effects of SAE on changes in the levels of SOD, Catalase, GPX and GSH in kidney tissue of normal and experimental rats.

Groups	SOD (U^1/mg protein)	Catalase (U^2/mg protein)	GPx (U^3/mg protein)	GSH (mg/100 g tissue)
Normal	2.83 ± 0.14[a]	2.10 ± 0.14[a]	7.25 ± 0.65[a]	50.16 ± 3.13[a]
Normal + SAE (150 mg/kg)	2.91 ± 0.11[a]	2.06 ± 0.18[a]	7.20 ± 0.76[a]	51.41 ±4.10[a]
NH4Cl (100 mg/kg)	1.62 ± 0.05[b]	1.35 ± 0.10[b]	4.0 ± 0.52[b]	22.37 ± 2.32[b]
NH4Cl + SAE	2.22 ± 0.12[c]	1.65 ± 0.11[c]	6.01 ± 0.67[c]	41.40 ± 3.52[c]
SAE control	2.80 ± 0.12[a]	2.08 ± 0.18[a]	7.21 ± 0.68[a]	50.42 ± 3.63[a]

Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscripts (a, b and c) differ significantly at $P < 0.05$ (DMRT). U^1 - one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 minute.
U^2 - µ mole of hydrogen peroxide consumed/minute. U^3- µg of glutathione consumed/min.

kidney through a peroxidative mechanism. In the liver, ammonia is removed either in the form of urea in periportal hepatocytes and/or as glutamine in perivenoushepatocytes[26]. Increased levels of blood ammonia might indicate hyperammonemic condition in rats treated with ammonium chloride, which may be due to liver damage caused by ammonia intoxication[27, 28]. The reduction in levels of ammonia during SAE treatment shows significant anti-hyperammonemic activity of this extract[29]. 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[30]. Decreased levels of blood ammonia and plasma urea in the SAE and NH_4Cl treated rats may be due to the antioxidant potential of SAE.

We observed that, NH_4Cl-induced rats exhibited decreased activities of SOD and catalase in kidney tissues. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of lipid peroxidation[31]. The decrease in the activities of these antioxidant enzymes might be due to damage of kidney tissues. SAE administration significantly normalized the activities of SOD and catalase in kidney tissues of NH_4Cl - induced rats and it was also reported that SAE possesses the ability to enhance the activity of SOD and catalase content in arthritic rats[32]. We observed decreased levels of GSH and GPx in kidney tissues of hyperammonemic rats; this might be due to the increased utilization in protecting 'SH'-containing proteins from lipid peroxides. SAE treatment significantly increased the levels of GSH and GPx in kidney tissues of NH_4Cl-induced rats; this might be due to SAE being able to increase the level of GSH and GPx in arthritic rats[32]. The present investigation shows that the SAE exerts protection to NH_4Cl-induced hyperammonemia in rats against oxidative stress. However, the exact mechanism is still unclear and further research on the action of SAE is underway.

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