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RESEARCH ARTICLE

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

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Abstract

To evaluate the protective 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, lipid peroxidation products in circulation and kidney tisssue and liver marker enzymes in serum of normal and experimental animals were analysed. Administration of SAE in hyperammonemic rats reduced the levels of ammonia, urea. The antioxidant property SAE was studied by assessing the activities of liver marker enzymes (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in NH_4Cl treated rats. Oxidative stress was effectively modulated by SAE administration. SAE significantly decreased the levels of ammonia, urea, lipid peroxidation products and liver marker enzymes as compared to NH_4Cl treated rats. This effect could be attributed to the presence of flavanoids, phenolic compounds, sterols and glycosides in *Semecarpus anacardium* nut.

Keywords: Hyperammonemia, *Semecarpus anacardium,* blood ammonia, liver marker enzymes

Introduction

Hyperammonemia is defined as an elevated ammonia concentration in blood, caused by an impairment of the liver function resulting in inadequate ammonia detoxification. 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[1]. Ammonia is a neurotoxin when accumulated in excess, and hyperammonemia is mainly responsible for the neurological alterations found in liver disease and hepatic encephalopathy, including impaired intellectual function[2]. Antiepileptic drugs, such as valproate and salicylate, cause hyperammonemia and urea cycle disorders[3,4]. Ammonia toxicity results in free radical generation that leads to oxidative stress-mediated tissue damage[5-7]. Presently available potent synthetic anti-hyperammonemic agents/therapies lie in their toxicity and the reappearance of symptoms after discontinuation[8]. 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[9]. SAE contains more than 20 active phyto-constituents. SAE was subjected to investigation against hepatocellular carcinoma[10],mammary carcinoma[11] and rheumatoid arthritis[9] in experimental mammals for its beneficial effects. Further, SAE is known to offer hepatoprotective[10], anti-inflammatory[12], antiatherogenic[13], cardio protective[14] and antiglycemic properties[15].

To our knowledge, this report is the first study to investigate the effect of SAE on circulation and kidney lipid peroxidation and liver marker enzymes 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 and plasma urea, thiobarbituric acid reactive substances (TBARS), hydroperoxides (HP), liver marker enzymes (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) 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[16].

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[17,18].

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)[9]; 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[19], Plasma urea[20], AST and ALT[21], ALP[22], TBARS[23] and HP[24].

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

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

The levels of TBARS, HP in kidney tissue and serum AST, ALT and ALP in normal and experimental rats are shown in Table 2. The levels of TBARS and HP in kidney tissue, liver markers in serum, were significantly higher in NH_4Cl-treated rats when compared with normal. Hyperammonemic rats treated with SAE significantly normalized the levels of lipid peroxidation products and liver markers, as compared with hyperammonemic rats.

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

Groups	Blood ammonia («mol/L)	Urea (mg/dl)	TBARS (nmol/ml)	HP (× 10^{-5} mmol/dl)
Normal	85.16±2.03[a]	12.58±0.78[a]	2.95±0.16[a]	8.15±0.75[a]
Normal + SAE(150 mg/kg)	84.16±0.68[a]	12.08±0.53[a]	2.85±0.28[a]	7.91±0.69[a]
NH_4Cl (100 mg/kg)	323.16±2.91[b]	24.83±0.55[b]	4.6±0.37[b]	14.15±0.93[b]
NH_4Cl+ SAE	145.0±1.91[c]	16.16±0.68[c]	3.4±0.22[c]	10.47±0.89[c]
SAE control	86.33±1.49[a]	12.41±0.67[a]	2.95±0.20[a]	8.62±0.85[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)

Table 2: Effects of SAE on changes in the levels of TBARS, HP in kidney tissue and the activities of serum liver marker enzymes (AST, ALT and ALP) of normal and experimental rats

Groups	Kidney TBARS (mM/100 g tissue)	Kidney HP (mM/100 g tissue)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal	1.40±0.1[a]	54.12±4.21[a]	75.48±1.68[a]	71.75±6.73[a]	24.58±2.32[a]
Normal + SAE (150 mg/kg)	1.45±0.1[a]	55.23±3.72[a]	73.11±1.62[a]	73.32±4.82[a]	25.95±1.52[a]
NH_4Cl (100mg/kg)	2.34±0.17[b]	76.43±5.68[b]	105.92±2.62[b]	134.83±2.50[b]	49.48±2.04[b]
NH_4Cl + SAE	1.83±0.13[c]	61.55±4.32[c]	84.58±1.76[c]	84.44±0.5[c]	32.21±0.87[c]
SAE control	1.5±0.15[a]	54.12±4.3[a]	71.93±1.1[a]	74.02±4.78[a]	23.42±2.49[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).

Discussion

In the liver, ammonia is removed either in the form of urea in periportal hepatocytes and/or as glutamine in perivenous hepatocytes[25]. 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[26]. The reduction in levels of ammonia during SAE treatment shows significant anti-hyperammonemic activity of this extract[28]. 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[29]. 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.

Our study shows the elevated levels of serum liver marker enzymes and lipid peroxidation products in circulation and kidney tissue of NH_4Cl treated rats might be due to the cell 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[30] due to increased Ca^{2+} concentration in the postsynaptic neuron[31]. Ca^{2+} binds to calmodulin and activates nitric oxide synthase, increasing the formation of NO that contributes to the neurotoxin process[32]. Decreased levels of liver marker enzymes and lipid peroxidation products in SAE administered rats may be due to its free radical scavenging property. Previous reports have shown that SAE is an effective free radical scavenger[9], lower lipid peroxidation in atherosclerosis[33], and have remarkable reduction in nitrate/nitrite level, which can be attributed to the antioxidant property[34,35].

In this investigation, increase in the level of plasma lipid peroxidation products in hyperammonemic conditions is generally thought to be the consequence of increased production and liberation of tissue lipid peroxides into circulation due to the pathological changes in tissues[17,26]. Treatment with SAE in hyperammonemic rats significantly decreased the levels of TBARS and HP in plasma. In our study, the increased activities of AST, ALT and ALP in serum obviously indicate that liver is susceptible to NH_4Cl induced toxicity and also this may be due to the liver damage caused by ammonia induced free radical generation. Treatment with SAE significantly decreased the levels of AST, ALT and ALP, suggesting that they offer protection by preserving the structural integrity of the hepatocellular membrane against ammonium chloride, and our findings are in agreement with the previously published results[28]. The present investigation shows that the SAE exerts protection to NH_4Cl-induced hyperammonemia in rats against oxidative stress.

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