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ORIGINAL ARTICLE

Protective influence of *Pongamia pinnata* (Karanja) on blood ammonia and urea levels in ammonium chloride-induced hyperammonemia: antihyperammonemic effect of the leaf extract

Musthafa Mohamed Essa, Perumal Subramanian, Ganapathy Suthakar, Tamilarasan Manivasagam, Kadiyala Babu Dakshayani

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India

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Summary
The present study was undertaken to investigate the antihyperammonemic efficacy of the leaf extract of *Pongamia pinnata*, an indigenous plant used in Ayurvedic Medicine in India (PPEt), on blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in control and ammonium chloride induced hyperammonemic rats. The levels of blood ammonia, circulatory urea, uric acid, non-protein nitrogen and creatinine increased significantly in rats treated with ammonium chloride and decreased significantly in rats treated with PPEt and ammonium chloride. There were no significant changes in the body weights of the experimental animals when compared to controls. The antihyperammonemic effect of PPEt could be attributed to (1) its nephroprotective effect by means of detoxifying excess urea and creatinine, (2) its free radical scavenging property, and (3) its antioxidant property. The exact mechanism of antihyperammonemic effect PPEt has still to be investigated and isolation of the active constituents is required.

Keywords: ammonium chloride – *Pongamia pinnata* – urea – uric acid – creatinine – non-protein nitrogen

INTRODUCTION

Hyperammonemia is a major contributing factor to neurological abnormalities observed in hepatic encephalopathy and in congenital defects of ammonia detoxication. Hyperammonemia is a heterogenous group of disorders characterized by elevated levels of ammonia, causing irritability, somnolence, vomiting, seizures, derangement of the cerebral function, coma and death (Tream 1994, Saez et al. 1999, Mathias et al. 2001, Murthy et al. 2001). Ammonia toxicity results in lipid peroxidation and free radical generation, which cause hepatic dysfunction and failure, significantly increases the number of brain peripheral benzodiazepine receptors and could also increase the affinity of ligands for these receptors that might enhance GABA adrenegergic neurotransmission.

✉ Perumal Subramanian, Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India
annamalai_rhythm@yahoo.co.in
☎ +91 4144 238343 ext: 210
+91 4144 238080

These changes probably contribute to deterioration of the intellectual function, decreased consciousness, coma and death (Hilgier et al. 1994, Kosenko et al. 1997, Lena and Subramanian 2004, Majeed 2005). The increased entry of ammonia to the brain is a primary cause of neurological disorders associated with hyperammonemia, such as hepatic encephalopathies, Reye syndrome, several other metabolic disorders, and some toxic encephalopathies. A five- to ten- fold increase in ammonia in the blood induces NMDA receptor mediated toxic effects in most animal species, with alterations in the function of the central nervous system (Majeed 2005).

The greatest disadvantage in presently available potent synthetic antihyperammonemic agents/therapies lies in their toxicity and the reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their antihyperammonemic activity is still in progress and there is much hope of finding antihyperammonemic drugs from indigenous medicinal plants. There is a need to search for appropriate protective agents against hyperammonemia. This can be focused on plants used in traditional medicine because of leads provided by natural products that may offer better treatment than currently used drugs.

Pongamia pinnata (L.) Pierre (Leguminosae, Papilionaceae; synonym *Pongamia glabra* Vent.), popularly known as 'Karanj' or 'Karanja' in Hindi, is a medium sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of South Eastern Asia and Australia (Krishnamurthi 1969, Satyavati et al. 1987). In the Ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments, and have been used in traditional medicines for bronchitis, whooping cough, rheumatic joints and to quench dipsia in diabetes (Kirtikar and Basu 1995). The seed and seed oil have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, and muscular and articular rheumatism (Nadkarni 1954). The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations (Kirtikar and Basu 1995). A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement (Chopra et al. 1933, Satyavati et al. 1987). Different extracts of roots and seeds (ethanol, petroleum, ether, benzene extracts and others) of *Pongamia pinnata* have been reported to have anti-inflammatory activity (Singh and Pandey 1996, Singh et al. 1996, Srinivasan et al. 2001). In addition, phytochemical examinations of this plant have indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, flavones, furanodiketones and flavonoid glucosides (Talapatra et al. 1980, 1982, Murthy and Seshadri 1944, Rangaswami et al. 1942, Sharma et al. 1973, Pathak et al. 1983, Toshiyuki et al. 1992, Ahmad et al. 2004).

However, despite the various phytochemical constituents and diverse medicinal activities attributed to this plant, no biochemical studies have been carried out to shed light on the role of *Pongamia pinnata* in hyperammonemia. In the light of the above, the present study was undertaken to investigate the effect of *Pongamia pinnata* leaf extract on blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in control and ammonium chloride treated rats.

MATERIALS AND METHODS

Plant Material

The mature green leaves of *Pongamia pinnata* (L.) Pierre were collected from Chidambaram, Cuddalore District, Tamil Nadu, India. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No.3670) was deposited in the Botany Department of Annamalai University.

Preparation of Alcoholic Extract (PPEt)

The shade-dried and powdered leaves of *Pongamia pinnata* were subjected to extraction with 70% ethanol under reflux for 8 h and concentrated to a semi-solid mass under reduced pressure (Rotavapor apparatus, Buchi Labortechnik AG, Switzerland). The yield was about 24% (w/ w) of the initial crude material (Srinivasan et al. 2001). In the preliminary phytochemical screening, the ethanolic extract of PPEt gave positive tests for glycosides, sterols, tannins and flavones (Trease and Evan 1959). The residual extract was dissolved in sterile water and used in the investigation.

Animals

Adult male albino Wistar rats, weighing 180-200g, bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. 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%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. All animal experiments were approved by the ethical committee, Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, India.

Hyperammonemia was induced in Wistar rats by daily intraperitoneal injections of ammonium chloride at a dose of 100 mg/kg body weight for

8 weeks (Velvizhi et al. 2002 a, b). In the experiment, a total of 32 rats were used. The rats were divided into 4 groups of 8 rats each. Group 1 rats were normal and untreated. Group 2 were normal rats treated with *Pongamia pinnata* leaf extract orally (300 mg/kg) (Srinivasan et al. 2001). Group 3 rats were treated with ammonium chloride intra peritoneally (100 mg/kg). Group 4 were rats treated with ammonium chloride (100 mg/kg) + *Pongamia pinnata* (300 mg/kg).

At the end of 8 weeks, all the rats were killed by decapitation after being given (Pentobarbitone sodium) anesthesia (60 mg/kg). Blood was collected for various biochemical estimations such as blood ammonia (Wolheim 1984), plasma urea by the diacetyl monooxime method, plasma uric acid by the Brown method, plasma non-protein nitrogen by Kjedhal's nesslerisation method and serum creatinine by the alkaline picrate method (Varley et al. 1998).

Statistical analysis

All data were expressed as mean ± S.D. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 9.5 (SPSS, Cary, NC, USA) and the individual comparison was done by Duncan's multiple range test (DMRT).

RESULTS

Ammonium chloride-treated rats showed increases in body weights compared to the control group. The ammonium chloride and PPEt-treated group and the PPEt alone treated group showed less significant differences in body weights when compared with the control and ammonium chloride treated group (Table 1). Concentrations of circulatory ammonia, urea, uric acid, non-protein nitrogen and creatinine (Table 1) increased significantly in ammonium chloride treated rats. Ammonium chloride and PPEt-treated rats showed significantly low levels of circulatory ammonia, urea, uric acid, non-protein nitrogen and creatinine when compared with the corresponding ammonium chloride group (Table 1). Rats treated with PPEt alone showed no significant differences in levels of ammonia, urea, uric acid, non-protein nitrogen and creatinine when compared with control rats.

DISCUSSION

Our results showed that rats treated with ammonium chloride gained significantly more body and tissue weight than did control rats. The increase might be due to increased levels of lipids during hyperammonemia. (Lena and Subramanian 2003, 2004, Velvizhi et al. 2002 a, b). Group 4 (PPEt with ammonium chloride) rats showed body weight gains similar to those in group 1 control rats, which might be due to the detoxifying, curative and preventive effects of PPEt (Shirwaikar et al. 2003).

In the liver, ammonia was removed either in the form of urea in periportal hepatocytes and/or as glutamine in perivenous hepatocytes (Nelson and Cox 2000). Increased levels of circulatory ammonia, urea, uric acid, non-protein nitrogen and creatinine might indicate a hyperammonemic condition in the rats treated with ammonium chloride (Lena and Subramanian 2003, 2004) and may be due to the liver damage caused by ammonia-induced free radical generation. Reports have shown that excess ammonia induces nitric oxide synthase, which leads to the enhanced production of nitric oxide, leading in turn to oxidative stress and liver damage (Kosenko et al. 2000, Schliess et al. 2002). Decreased levels of blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in the PPEt and ammonium chloride treated rats may be due to the antioxidant potential of PPEt, and also it has been reported that *Pongamia pinnata* flower extract normalizes the levels of urea and creatinine during cisplatin induced nephrotoxicity (Shirwaikar et al. 2003). Our present findings corroborate these reports. Previous reports have shown that PPEt is an effective free radical scavenger (Prabha et al. 2003) and also that *in vitro* studies of the alcoholic extract of PP flowers have revealed marked nitric oxide scavenging activity, suggesting a potent antioxidant property (Shirwaikar et al. 2003).

A relationship between oxidative stress and hyperammonemia has been well established and evidences point to the fact that ammonium (acetate / chloride) salts induce hyperammonemia partly via oxidative stress (Kosenko et al. 1997, Dakshayani et al. 2002 a, b, Velvizhi et al. 2002 a, b, Vidhya and Subramanian 2003, Lena and Subramanian 2003, 2004). Flavanoids are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules (Devipriya and Shyamaladevi 1999). The plant is known to contain a number of bioflavonoids like kaempferol, quercetin, karanjin, kanjone, pongaglabrone, gammatin, pongaglabol, kanugin etc. (Satyavati et al. 1987).

Phytochemicals such as quercetin, kaempferol etc. are well known potent free radical scavengers and also it has been reported that the root extract of *Pongamia pinnata* tends to reverse the change in lipid peroxidation activity, indicating decreased lipid peroxidation and damage to cells (Prabha et al. 2003). Hence, the mechanism by which the PPEt exerts an antihyperammonemic effect could be

attributed to its nephroprotective effect by means of detoxifying excess urea and creatinine, free radical scavenging and antioxidant properties. The exact mechanism of the antihyperammonemic effect has to be still investigated and isolation of the active constituents is required.

Table 1. **Effect of PPEt on changes in the body weight, blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine of normal and experimental rats**

Group	Changes in body weight (g)		Blood ammonia (μmol/L)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Non protein nitrogen (mg/dl)
	Initial	Final					
Normal	180.03 ± 13.71	194.03 ± 14.77[a]	88.28 ± 6.72 [a]	10.95 ± 0.83 [a]	1.72 ± 0.13 [a]	0.81 ± 0.06 [a]	23.85 ± 1.82 [a]
Normal + PPEt (300 mg/kg)	189.03 ± 14.39	196.03 ± 14.93[a]	83.93 ± 6.39 [a]	11.50 ± 0.88 [a]	1.73 ± 0.13 [a]	0.86 ± 0.07 [a]	24.05 ± 1.83 [a]
NH_4Cl treated (100 mg/kg)	186.03 ± 14.17	204.03 ± 15.54[a]	331.21 ± 25.22 [b]	21.80 ± 1.66 [b]	2.29 ± 0.17 [b]	1.31 ± 0.10 [b]	50.96 ± 3.88 [b]
NH_4Cl treated (100 mg/kg) + PPEt (300 mg/kg)	185.03 ± 14.09	198.03 ± 15.08 [a]	166.79 ± 12.70 [c]	13.09 ± 1.00 [c]	1.79 ± 0.14 [a]	0.89 ± 0.07 [a]	30.09 ± 2.29 [c]

Values are given as mean ± S.D from six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)
Duncan procedure; Ranges for the levels: 2.91; 3.06; 3.16; 3.22.

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