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Original article

Effect of *Pongamia pinnata* leaves on serum lipids in ammonium chloride induced experimental hyperammonemic rats

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ABSTRACT

Effect of *Pongamia pinnata* (an indigenous plant used in Ayurvedic Medicine in India) leaf extract (PPEt) on the levels blood ammonia, and serum lipid profiles (cholesterol, triglycerides, phosphor lipids, free fatty acids) were studied for its protective effect during ammonium chloride induced hyperammonemia in Wistar rats. Ammonium chloride (AC) treated rats showed a significant increase in the levels of circulatory ammonia and lipid profiles. These changes were significantly decreased in PPEt and AC treated rats. Our results indicate that PPEt offers protection by influencing the levels of ammonia and lipid profiles in experimental hyperammonemia and this could be due to its (i) ability to detoxify excess ammonia, urea and creatinine, (ii) free radical scavenging property both in vitro and in vivo by means of reducing lipid peroxidation and the presence of natural antioxidants. Hence, it may be concluded that the hypolipidaemic and antihyperlipidaemic effects produced by the PPEt may be due to the presence of flavonoids and other polyphenolic compounds. But the exact underlying mechanism is remains to be elucidated.

1. Introduction

Hyperammonemia is a major contributing factor to neurological abnormalities observed in hepatic encephalopathy and in congenital defects of ammonia detoxication [1]. Ammonia is a neurotoxin that has been strongly implicated in the pathogenesis of hepatic encephalopathy [2]. 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 [3]. It was reported that elevated levels of ammonia causing irritability, somnolence, vomiting, seizures, derangement of cerebral function, coma and death [1, 4]. Ammonia toxicity results in lipid peroxidation and free radical generation, which cause hepatic dysfunction and failure and significantly increase number of brain peripheral benzodiazepine receptors and could increase the affinity of ligands for these receptors that might enhance GABA (gamma amino butyric acid) adrenegergic neurotransmission. These changes probably contribute to deterioration of intellectual function, decreased consciousness, coma and death [5-7].

In recent years of scientific investigations, attention has been drawn to the health promoting activity of plant foods and its active components. *Pongamia pinnata* (Linn) Pierre is a medium sized glabrous tree popularly known as Karanja in Hindi, Indian Beech in English, and Pungai in Tamil. Different parts of this plant have been used in Ayurvedic medicine for bronchitis, whooping cough, rheumatic joints and quench dipsia in diabetes [8]. Most of the Tamil Nadu physicians of Indian system of traditional medicine Ayurveda and Siddha use *P. pinnata* to treat various kinds of diseases including leucoderma, leprosy, lumbago, muscular, articular rheumatism [9] and diabetes mellitus. The leaves are anthelmintic and cure piles, wounds and other inflammations [10]. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement. Different extracts of roots and seeds of *P. pinnata* have been reported to have anti-inflammatory and antiulcer activities [11]. To our best knowledge no scientific data regarding the hypolipidaemic and antihyperlipidaemic effect of *P. pinnata* leaves during hyperammonemia are available except in the treatise of Ayurvedic medicine. Thus, the present study was undertaken to evaluate the protective effect of ethanolic extract of

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P. pinnata leaves on lipid profile in ammonium chloride induced experimental hyperammonemic Wistar rats.

2. Materials and Methods

2.1. Animals

Adult male albino Wistar rats, weighing 180-200 g 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%. Animals were fed with a standard pellet 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.

2.2. Plant Material and preparation of Extract (PPEt)

The mature green leaves of *P. pinnata* 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. The shade-dried and powdered leaves of *P. 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 starting crude material. In the preliminary phytochemical screening, the ethanolic extract of PPEt gave positive tests for glycosides, sterols, tannins and flavones [12]. The residual extract was dissolved in sterile water and used in the investigation. Ammonium chloride was purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals used in the study were of analytical grade. Hyperammonemia will be induced in Wistar rats by daily intraperitoneal injections of ammonium chloride at a dose of 100 mg/kg body weight for 8 weeks [13].

2.3. Experimental design

In this experiment, a total of 24 rats were used. The rats were divided into 4 groups of 6 rats each. Group I rats were normal untreated. Group II were normal rats treated with PPEt orally (300 mg/kg bodyweight) [7, 11, 13]. Group III rats were treated with ammonium chloride intra peritoneally (100 mg/kg bodyweight) [7, 13]. Group IV were rats treated with ammonium chloride + PPEt. At the end of 8 weeks, all the rats were killed by decapitation after giving (Pentobarbitone sodium) anesthesia (60 mg/kg). Blood samples were collected for various biochemical estimations.

Blood ammonia levels were estimated by the method of Wolheim [14]. Circulatory Cholesterol levels were analyzed by the method of Zlatkis [15]. Serum triglycerides were analyzed by method of Foster and Dunn [16]. Phospholipids were analysed by the method of Zilversmit and Davis [17] and the serum free fatty acids were estimated by the method of Falholt [18].

2.4.Statistical analysis

Statistical analysis was carried out by analysis of variance (ANOVA) and the groups were compared using Duncan's Multiple Range Test (DMRT).

3. Results

Table 1 shows the levels of blood ammonia of control and experimental animals. Circulatory ammonia levels increased significantly and the levels reduced significantly in ammonium chloride and PPEt treated rats. Normal rats treated with PPEt showed no significant differences in levels of ammonia when compared with control rats (Table 1).The effect of PPEt on the levels of serum cholesterol, triglycerides, free fatty acids and phospholipids in normal and experimental groups illustrated in Table 1. The levels of all these lipids were significantly increased in AC induced hyperammonemic rats whereas the administration of PPEt to AC treated rats significantly restored all these changes to almost normal levels (Table 1).

Table 1. Effect of PPEt on changes in the blood ammonia and serum lipid profiles of normal and experimental rats

Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Free fatty acids (mg/dl)	Phospholipids (mg/dl)	Blood ammonia (mol/L)
1. Normal	84.61 4.03[a]	60.17 3.60[a]	73.2 3.95[a]	101.6 5.2[a]	88.28 ± 6.72[a]
2.Normal + PPEt	82.17 3.59[a]	56.95 3.01[a]	69.3 3.64[a]	96.8 4.71[a]	83.93 ± 6.39[a]
3.AC treated	171.25 12.5[b]	91.85 5.45[b]	149.2 7.92[b]	171.1 11.7[b]	331.21 ± 25.22[b]
4.AC + PPEt	121.20 10.7[c]	67.84 4.01[c]	92.1 5.13[c]	121.3 6.9[c]	139.79 ± 10.70[c]

Values are given as mean S.D from six rats in each group
ANOVA followed by Duncan's multiple range tests Values not sharing a common superscript (a, b, c) differ significantly at $p \leq 0.05$

4. Discussion

Ammonia is removed either in the form of urea in periportal hepatocytes and/or as glutamine in perivenous hepatocytes in liver [19]. An increased level of circulatory ammonia might indicate a hyperammonemic condition in the rats treated with ammonium chloride [6, 7, 13, 20]. Decreased levels of blood ammonia, PPEt and ammonium chloride treated rats show the significant anti-hyperammonemic activity of this plant and it was reported that *P. pinnata* normalized the levels of ammonia, urea and creatinine during hyperammonemic and nephrotoxic conditions [7, 9, 13]. Our present findings have an in agreement with these reports and the exact mechanism remains to be explored.

Oxidative stress mediated lipid peroxidation was also shown as one of the characteristic features of hyperammonemia [6, 7, 21]. Free radical damage to cellular components and decomposition of hydroperoxide formed from oxidative breakdown of polyunsaturated fatty acids (PUFAs) are important factors in the development of cellular toxicity and pathology caused by lipid peroxidation. In a large number of tissues, it is now generally

In the present study, elevated levels of ammonia caused a significant rise in serum lipids (cholesterol, triglycerides, phospholipids and free fatty acids) were observed. These findings indicate that hyperammonemia may be accompanied by complications of atherosclerosis. Previous studies from our lab reported that the levels of serum and tissue lipid were elevated during hyperammonemic conditions [6, 20, 23, 24]. Previous studies have indicated that viral infections, aspirin treatment and hyperammonemia are associated with Reye's syndrome. It has also been reported that free fatty acids in serum and total lipids in the liver of Reye's syndrome patients are elevated during illness [25].

It was reported that ammonium (chloride/acetate) salts may deplete levels of α-KG and other Krebs cycle intermediates [6, 7, 23, 26, 27] and thus elevate the levels of acetyl coenzyme A. The elevated levels of acetyl coenzyme A may increase levels of lipid profile (free fatty acids, triacylglycerols, phospholipids, and cholesterol), as observed in our study. Another important function of α-KG occurs in the formation of carnitine [6, 7, 19, 24, 26, 27]. Carnitine acts as a carrier of fatty acids into cell mitochondria so that proper catabolism of fats can proceed [19, 23, 26, 27]. The decreased α-KG and other Krebs cycle intermediates levels in rats treated with AC might have led to accumulation of fatty acids [23].

Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of various vascular diseases [28]. In the present study, PPEt treatment to hyperammonemic rats caused a significant decrease in serum lipids (cholesterol, triglycerides, phospholipids and free fatty acids). The effect of PPEt on controlled mobilization of serum cholesterol, triglycerides, phospholipids and free fatty acids presumably mediated possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. Previous reports showed that the leaf extract have the ability to normalize the levels of serum and tissue lipids during diseased conditions [10].

In conclusion, the alterations in serum lipids during experimental hyperammonemia were restored to near normal levels by PPEt treatment. Phytochemical studies of *P. pinnata* extract revealed the presence of flavonoids and other polyphenolics in various concentrations. Several authors reported that, flavonoids and phenolic compounds have hypolipidaemic and antihyperlipidaemic effects [29, 30]. Hence, it may be concluded that the hypolipidaemic and antihyperlipidaemic effects produced by the PPEt may be due to the presence of flavonoids and other polyphenolic compounds.

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