ANTIHYPERTENSION EFFECT OF WITHANIA SOMNIFERA ON AMMONIUM CHLORIDE INDUCED WISTAR RATS

HARIKRISHNAN, B., SUBRAMANIAN, P.² AND SUBASH, S.

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, India. E. mail: annamalai_rhythm@yahoo.co.in

Received: December 26, 2007; Revised: April 12, 2008; Accepted: April 14, 2008

Abstract: The present study was undertaken to investigate the antihyperammonemic efficacy of Withania somnifera root, which has been used in Indian traditional medicine for many centuries. We studied the effect of oral administration of powdered root of W. somnifera (500 mg/kg; b.wt) on blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in control and ammonium chloride (AC) induced hyperammonemic rats. The levels of circulatory ammonia, urea, uric acid, non-protein nitrogen and creatinine decreased significantly in rats treated with W. somnifera and AC. These studies indicate that powdered root of W. somnifera may offer protection against AC induced hyperammonemia. The antihyperammonemic effect of W. somnifera may be due to (i) the presence of alkaloids, withanolids and flavonoids, (ii) normalizing the levels of urea and urea related compounds, (iii) its free radical scavenging property and (iv) its antioxidant property. The exact underlying mechanism is still unclear and further research needed.

Key words: Ammonium chloride, Withania somnifera

INTRODUCTION

Hyperammonemia is a heterogenous group of disorders characterized by elevated levels of ammonia causing irritability, somnolence, vomiting, seizures and derangement of cerebral function, coma and death [1-3]. 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 [4]. Ammonia is also a major pathogenetic factor associated with inborn errors of urea cycle, Reye’s syndrome, organic acidurias and disorders of fatty acid oxidation [5]. Ammonia-induced neurotoxicity has been reported to include a dysfunction of multiple neurotransmitter system including glutamate mediated excitotoxicity, electrophysiological disturbances and defects in brain bioenergetic [6]. In spite of extensive investigations, the precise mechanism(s) involved in ammonia neurotoxicity has not been completely understood.

There is a need to search for appropriate protective agents against hyperammonemia without side effects. This search can be focused on plants used in traditional medicine and on natural products that may offer treatment for hyperammonemia than currently used drugs. Ashwagandha (Withania somnifera L. Dunal solanaceae) is an important medicinal plant, widely used as a home remedy for several diseases in India as well as other parts of the world [7].

The chemical composition, pharmacological and therapeutic efficacy of this plant has been well established [8]. Different investigators reported that W. somnifera possess antiserotogenic, hyperlipidemia, Parkinson’s disease [9], anticancer, anabolic properties and has beneficial effects in the treatment of arthritis, geriatric problems [10] and stress [11]. Further the plant has been reported to have anti-inflammatory, antitumour, antistress, antioxidant, immunomodulatory, hematopoietic and rejuvenating...
properties [12,13]. It is one of the most commonly used herbs, not only as an antistress and adaptogenic agent, but is also known to increase life span and delay ageing [14]. The roots of *W. somnifera* contain several alkaloids, withanolides, a few flavonoids and reducing sugars [15] and are also rich in iron [16]. The major active compounds of the roots are reported to be withanolides, glycosides and many different alkaloids. To date, up to 19 withanolide derivatives have been isolated from *Withania* roots [17].

However, despite the observation of diverse medicinal properties attributed to this plant, no biochemical studies have been carried out to shed light on the role of *W. somnifera* on hyperammonemia. In this context, the present study was undertaken to investigate the effect of powdered root of *W. somnifera* on blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in control and ammonium chloride induced hyperammonemic rats.

**MATERIALS AND METHODS**

**Plant material:** The dried roots of *W. somnifera* were collected from Chidambaram, Cuddalore District, Tamil Nadu. The roots were identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No: 2934) was deposited in the Botany Department of Annamalai University.

**Preparation of *W. somnifera***: The roots were thoroughly washed with 95% ethanol and with sterile water. They were air dried and powdered. The powder was dissolved in sterile water and used in the investigation.

**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%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water and *ad libitum*. All experiments were approved by the ethical committee (vide No.355/2006, date: 18.10.2006), Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, India.

**Experimental design:** Hyperammonemia was induced in Wistar rats by intraperitoneal injections of ammonium chloride (AC) at a dose of 100 mg/kg body weight thrice in a week for 8 consecutive weeks [3]. In the experiment, a total number of 32 rats were used. The rats were divided into 4 groups of 8 rats each. Group I rats were normal anduntreated. Group II normal rats were administered with *W. somnifera* (500 mg/kg body weight) using an intragastric tube [18], group III rats were treated with AC (100 mg/kg body weight; i.p) and group IV rats were treated with AC (100 mg/kg) + *W. somnifera* (500 mg/kg) thrice in a week for 8 weeks. At the end of 8 weeks, the rats were kept overnight fasting and sacrificed by cervical dislocation after anaesthetizing the animal with intramuscular injection of ketamine hydrochloride (30 mg/kg body weight). Blood was collected for various biochemical estimation such as blood ammonia [19], plasma urea (Diacetyl monoxime method), plasma uric acid, plasma non-protein nitrogen (Kjeldahl’s method) and serum creatinine by the alkaline picrate method [20].

**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 8 rats in each group. p values <0.05 were considered as significant.

**RESULTS**

Table 1 shows changes in the levels of body weight, circulatory ammonia, urea, uric acid, non-protein nitrogen and creatinine in control and experimental rats. There is no significant change in the body weight of the control and experimental rats. The levels of ammonia, urea, uric acid, non-protein nitrogen and creatinine increased significantly in the AC treated rats. Administration of *W. somnifera* to the hyperammonemic rats significantly reduced the levels of ammonia, urea, uric acid, non-protein nitrogen and creatinine when compared with the corresponding AC treated group. Rats treated with *W. somnifera* alone showed no significant differences in levels of ammonia, urea, acid, non-protein nitrogen and creatinine when compared with the control rats.

**DISCUSSION**

In the liver, ammonia was removed either in the form of urea in periportal hepatocytes and/or as glutamine.
in perivenous hepatocytes [21]. Increased levels of circulatory ammonia, urea, uric acid, non-protein nitrogen and serum creatinine might indicate a hyperammonemic condition in the rats treated with AC [22] and may be due to the liver damage caused by ammonia-induced free radical generation. Reports have shown that excess ammonia induces nitric oxide synthesis, which leads to the enhanced production of nitric oxide, leading in turn to oxidative stress and liver damage [2]. Decreased levels of blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in W. somnifera and AC treated rats may be due to the antioxidant potential of W. somnifera and it was reported that plant products, phenolic compounds and flavonoids have the ability to remove excess ammonia, urea, uric acid, non-protein nitrogen and creatinine and offer protection against hyperammonemic and nephrotic conditions [23] and this plant has antioxidant, antiperoxidative and free radical quenching properties in various diseased conditions [24].

The roots of W. somnifera contain several alkaloids, withanolides, a few flavonoids and reducing sugars [25]. The active compounds reported in W. somnifera include withaferin A, 1-oxo-5β and 6β-epoxy-witha-2-ene-27-enoxy-olide [25,26]. These reports suggest that Withania is a rich source of bioactive compounds [17]. Phytochemicals are well known potent free radical scavengers and it has also been reported that the root extract of W. somnifera tends to reverse the changes in lipid peroxidation and damage to cells [8]. Hence, the mechanism by which the W. somnifera exerts an antihyperammonemic effect could be attributed to (i) presence of natural antioxidants, (ii) its free radical scavenging and antioxidant properties and (iii) removal of excess urea related compounds. The exact underlying mechanism is still unclear and further research is needed.

ACKNOWLEDGEMENTS

B. Harikrishnan, gratefully acknowledges the financial assistance in the form of “Research Student Fellowship” from Annamalai University.

REFERENCES