

ORIGINAL ARTICLE

Influence of *Hibiscus sabdariffa* (Gongura) on the levels of circulatory lipid peroxidation products and liver marker enzymes in experimental hyperammonemia

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Received 20th September 2005.

Published online 15th November 2005.

Summary

Hibiscus sabdariffa (Linn) (family Malvaceae), is an annual dicotyledonous herbaceous shrub plant popularly known as ‘Gongura’ in Hindi or ‘Pulicha keerai’ in Tamil, which is an indigenous edible medicinal plant used in Ayurvedic Medicine in India, China and Thailand. We have investigated the influence of *Hibiscus sabdariffa* leaf extract (HSEt) on the levels of circulatory ammonia, urea, lipid peroxidation products such as TBARS (thiobarbituric acid and reactive substances), HP (hydroperoxides) and liver marker enzymes such as AST (aspartate transaminase), ALT (alanine transaminase) and ALP (alkaline phosphatase), for its hepatoprotective effect in ammonium chloride induced hyperammonemia. Ammonium chloride treated rats showed a significant increase in the levels of circulatory ammonia, urea, AST, ALT, ALP, TBARS and HP. These changes were significantly decreased in rats treated with HSEt and ammonium chloride. Our results indicate that HSEt offers hepatoprotection by influencing the levels of lipid peroxidation products and liver markers in experimental hyperammonemia and this could be due to its free radical scavenging property and the presence of natural antioxidants. The exact mechanism has to be still investigated and the isolation of active constituents is required.

Key words: ammonium chloride – hyperammonemia – urea – lipid peroxidation – AST – ALT – ALP

INTRODUCTION

Hyperammonemia is a major contributing factor to neurological abnormalities observed in hepatic

encephalopathy and in congenital defects of ammonia detoxication (Rodrigo et al. 2004). Hyperammonemia is a heterogenous group of disorders characterized by elevated levels of ammonia, causing irritability, somnolence, vomiting, seizures, and derangement of the cerebral function, coma and death (Tream 1994, Saez et al. 1999, Mathias et al. 2001, Murthy et al. 2001 Rodrigo et al. 2004). Ammonia toxicity results in lipid peroxidation and free radical generation, which cause hepatic dysfunction and failure and significantly increase the number of brain peripheral benzodiazepine receptors and could increase the affinity of ligands for these receptors that might enhance GABA (gamma-amino butyric

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acid) adrenergic neurotransmission. These changes probably contribute to the deterioration of intellectual function, decreased consciousness, coma and death (Hilgier et al. 1994, Kosenko et al. 1997, Lena and Subramanian 2004, Majeed 2005). 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 tenfold 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 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 for finding antihyperammonemic drugs from indigenous medicinal plants. There is a need for the 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.

Hibiscus sabdariffa (Linn) (family Malvaceae), is an annual dicotyledonous herbaceous shrub plant popularly known as 'Gongura' in Hindi or 'Pulichha keerai' in Tamil. This plant is well known in Asia and Africa and is commonly used to make jellies, jams and beverages. In the Ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments like hypertension, pyrexia and liver disorders. In traditional medicine, this plant has good features useful in several applications, such as antidotes to poisonous chemicals (acids, alkali, pesticides) and venomous mushrooms (Chifundera et al. 1994). Previous phytochemical investigations of this plant show the presence of phenolic compounds, anthocyanins, flavonols, protocatechuic acid (PCA), etc. (Seca et al. 2000, 2001). It is a well-documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties (Shirwaikar et al. 2003). Recently, anthocyanin and PCA, have been found to have antioxidant activity, and to offer protection against atherosclerosis and cancer (Meyer et al. 1997, Satue-Gracia et al. 1997). Anthocyanins were also found to have many times more activity than common antioxidants such as ascorbate (Wang, et al. 1997). Overall, there is now increasing evidence that antioxidants in the human diet are of major benefit for health and well-being.

However, there are various phytochemical constituents and diverse medicinal activities attributed to this plant and no biochemical studies have been carried out to shed light on the role of *Hibiscus sabdariffa* on the levels of lipid peroxidation products and liver marker enzymes in experimental hyperammonemia. In the light of the above, the present study was undertaken to investigate the effect of *Hibiscus sabdariffa* leaf extract on blood ammonia, plasma urea, TBARS (thiobarbituric acid and reactive substances), HP (hydroperoxides) and the liver marker enzymes such as AST (aspartate transaminase), ALT (alanine transaminase) and ALP (alkaline phosphatase) in serum in control and ammonium chloride treated rats.

MATERIALS AND METHODS

Plant Material

The mature green leaves of *Hibiscus subdariffa* 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.3648) was deposited in the Botany Department of Annamalai University.

Preparation of Alcoholic Extract (HSEt)

The shade-dried and powdered leaves of *Hibiscus subdariffa* 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 (Srinivasan et al. 2001, Essa et al. 2005). In the preliminary phytochemical screening, the ethanolic extract of HSEt gave positive tests for glycosides, anthocyanins, polyphenols and flavones (Trease and Evan 1959). The residual extract was dissolved in sterile water and used in the investigation.

Chemicals

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

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 $22 \pm 2^\circ\text{C}$ 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.

Induction of experimental hyperammonemia

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 (Essa et al. 2005).

Experimental design

In the experiment, a total of 32 rats were used. The rats were divided into 4 groups of 8 rats each. Group I rats were normal and untreated. Group II were normal rats treated with HSEt orally (250 mg/kg bodyweight) (Odigie et al. 2003). Group III rats were treated with ammonium chloride intra peritoneally (100 mg/kg bodyweight) (Essa et al. 2005). Group IV were rats treated with ammonium chloride (100 mg/kg) + HSEt (250 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 samples were collected for various biochemical estimations such as blood ammonia (Wolheim 1984), plasma urea (Varley et al. 1998), plasma TBARS (Niehaus and Samuelson 1968), plasma HP (Jiang et al. 1992), serum AST and ALT (Reitman and Frankel 1957) and serum ALP (King and Armstrong 1934).

Statistical analysis

Statistical analysis was carried out by analysis of variance (ANOVA) and the groups were compared using Duncan's Multiple Range Test (DMRT) at the significance level $2\alpha=0.05$.

RESULTS

The levels of circulatory ammonia, urea, TBARS, HP, AST, ALT and ALP increased significantly in ammonium chloride treated rats (Table 1). Ammonium chloride and HSEt-treated rats showed significantly low levels of circulatory ammonia, urea, TBARS, HP, AST, ALT and ALP when compared with the corresponding ammonium chloride group (Table 1). Rats treated with HSEt alone showed no significant differences in levels of ammonia, urea, TBARS, HP, AST, ALT and ALP when compared with control rats (Table 1).

DISCUSSION

In the liver, ammonia is 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 and urea might indicate an hyperammonemic condition in the rats treated with ammonium chloride (Velvizhi et al. 2002 a, b, Lena and Subramanian 2003, 2004, Essa et al. 2005). Increased levels of circulatory TBARS, HP, AST, ALT and ALP may be due to the liver damage caused by ammonia-induced free radical generation. Reports have shown that excess ammonia induces nitric oxide synthase, leading to enhanced production of nitric oxide, which in turn leads to oxidative stress and liver damage (Kosenko et al. 2000, Schliess et al. 2002). Decreased levels of circulatory ammonia, urea, TBARS, HP, AST, ALT and ALP in group IV rats may be due to the free radical scavenging property of HSEt (Liu et al. 2002, Amin and Hamza 2005). Previous reports show that alcoholic extract of HS flowers and calyces revealed marked nitric oxide scavenging activity (Obiefuna et al. 1993, Adegunloye et al. 1996, Wang et al. 2000, Liu et al. 2002, Odigie et al. 2003, Amin and Hamza 2005). It was reported that phenolic compounds and flavonoids have the ability to remove excess ammonia and urea and offer protection against hyperammonemia (Essa et al. 2005), which corroborates our present findings.

A relationship between oxidative stress and hyperammonemia has been well established and evidence indicates 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, Essa et al. 2005). Flavanoids and polyphenolic compounds are potent free radical scavengers 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 such as anthocyanins, glycosides, PCA, hydroxycitric acid, etc. (Lewis and Neelakantan 1965, Osman et al. 1975). Phytochemicals such as anthocyanin, flavonols, glycosides, etc. are well known potent free radical scavengers and also it was reported that the calyces extract of *Hibiscus sabdariffa* tend to reverse the change in lipid peroxidation activity, indicating decreased lipid peroxidation and damage to cells and overall, HS is a good source of antioxidants (Tseng et al. 1996, Duh and Yed 1997, Tsai et al. 2002). Hence, the possible mechanism by which the HSEt exerts a hepatoprotective effect in a hyperammonemic condition could be attributed to the presence of natural antioxidants and its free radical scavenging properties. The exact mechanism has to be still investigated and isolation of the active constituents is required.

Table 1. Effect of HSEt on changes in the blood ammonia, plasma urea, TBARS, HP, serum AST, ALT and ALP of control and treated rats

Group	Liver marker enzymes			Blood ammonia ($\mu\text{mol/L}$)	Blood Urea (mg/dl)	TBARS (nmol/ml)	HP ($\times 10^{-5}$ mmol/dL)
	AST (IU/L)	ALP (IU/L)	ALT (IU/L)				
Control	72.49 \pm 5.21 ^a	75.51 \pm 5.53 ^a	25.16 \pm 2.31 ^a	88.01 \pm 6.70 ^a	10.79 \pm 0.82 ^a	1.53 \pm 0.092 ^a	8.40 \pm 0.78 ^a
Control + HSEt (250 mg/kg)	69.43 \pm 5.17 ^a	72.31 \pm 4.87 ^a	23.45 \pm 2.23 ^a	82.30 \pm 6.27 ^a	11.60 \pm 0.88 ^a	1.51 \pm 0.082 ^a	8.19 \pm 0.54 ^a
Ammonium chloride treated (100 mg/kg)	114.29 \pm 7.47 ^b	138.72 \pm 9.12 ^b	61.34 \pm 4.24 ^b	342.67 \pm 26.09 ^b	22.18 \pm 1.69 ^b	3.03 \pm 0.230 ^b	14.41 \pm 1.29 ^b
Ammonium chloride (100 mg/kg) + HSEt (250 mg/kg)	82.98 \pm 6.42 ^c	86.50 \pm 6.31 ^c	29.99 \pm 2.78 ^c	163.70 \pm 12.46 ^c	13.00 \pm 0.99 ^c	1.81 \pm 0.139 ^c	9.70 \pm 0.55 ^c

ANOVA followed by Duncan's multiple range test

Values not sharing a common superscript (a, b, c) differ significantly at $2\alpha=0.05$

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