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Fisetin, a dietary flavonoid, attenuates hyperammonemia and improves circadian locomotor deficits, redox balance, and astrocytic markers in rats

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ABSTRACT

Fisetin, a bioactive flavonoid, found in many fruits, was administered orally (50 mg/kg body weight) to hyperammonemic rats (treated with ammonium chloride (AC) for 8 weeks (i.p. injections, 100 mg/kg body weight). Treatment with AC augmented oxidative stress, levels of ammonia, enzymes (alanine and aspartate transaminases and alkaline phosphatase) in blood, glutamate and glutamine content in brain, caused disturbances in the circadian locomotor rhythm with a reduction in amplitude and period and downregulated the expression of soluble guanylyl cyclase (sGC) and glial fibrillary acidic protein (GFAP) in brain. Co-treatment of fisetin treatment with AC, prevented the AC-induced elevation in oxidative stress, normalized the levels of ammonia, transaminases and alkaline phosphatase in circulation, glutamate and glutamine in brain, upregulated the expression of sGC and GFAP and stabilized the circadian locomotor rhythm. Thus, fisetin assuages AC induced effects through its antioxidant, anti-inflammatory, cytoprotective, neuroprotective and neurotrophic properties.

1. Introduction

Fisetin (3, 3′, 4′ 7 – tetrahydroxyflavone), a flavonoid, extensively dispersed in fruits (grapes, apples, strawberries and persimmons) at concentrations of 2–160 mg/g (Scalbert & Williamson, 2000), has been reported to possess antioxidant (Sengupta, Banerjee, & Sengupta, 2004), cancer preventing (Sung, Pandey, & Aggarwal, 2007), allergy preventing (Kim et al., 2014), anti-inflammatory (Tago, Nakamura, Tago, Mashino, & Kasahara, 2011), antidiabetic (Maher et al., 2011), neuroprotective (Chiruta, Schubert, Dargusch, & Maher, 2012), neurotrophic (Maher,

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2009), and antiangiogenic (Fotsis et al., 1997) properties. Fisetin counters osteoporosis and supplementation of fisetin significantly thwarts bone loss and inflammation in mice (Leotoing et al., 2013). Fisetin is reported to enhance memory in rats (Maher, 2009), offers defensive capacity against oxidative glutamate toxicity and thus preventing peroxynitrite-mediated damages in primary rat neurons (Ishige, Schubert, & Sagara, 2001). Flavonoids, being structurally analogous to vitamin E, could mimic their antioxidant action in cell membranes (Acker, Schouten, Haenen, Vijgh, & Bast, 2000). The 3-OH group of fisetin has the lowest estimated BDE (bond dissociation energy) value (O—H covalent bond) succeeded by its 3′- and 4′-OH groups. A lower BDE value is attributed to a higher ability to donate a hydrogen atom from the hydroxyl group and thereby to scavenge free radicals (Cao, Sotic, & Prior, 1997). Fisetin and its derivatives could enhance intracellular glutathione (GSH) levels and maintenance of GSH levels is linked with cell survival with a multiplicity of paradigms (Touil et al., 2011).

The prevalence of hyperammonemia and urea cycle disorders globally is estimated at 1 per 30,000 live births (Msall, Batshaw, Suss, Brusilow, & Mellits, 1984). Elevated ammonia during hyperammonemic conditions affects several important central nervous system (CNS) functions including energy metabolism, hemodynamics and neurotransmission (Bosoi & Rose, 2009). Liver failure or disease reduces the capacity of the liver to convert ammonia into urea and leads to hyperammonemia. Hyperammonemia, in turn, causes altered glutamatergic neurotransmission (Rodrigo & Felipo, 2006), hepatic encephalopathy (HE) and a spectrum of neuropsychiatric and neurological symptoms with high mortality (Felipo & Butterworth, 2002).

Ammonia intoxication also impairs mitochondrial function, decreases ATP synthesis and increases the formation of free radicals (Kosenko, Venediktova, Kaminsky, Montoliu, & Felipo, 2003). Pathogenesis of hyperammonemia is associated with free radical induced oxidative/nitrosative damage (Kosenko et al., 2003). The potential association of oxidative stress in addition to nitrosative stress in contributing to the deleterious effects of ammonia has been documented (Murthy, Rao, Bai, & Norenburg, 2001). Hyperammonemia elevates brain glutamine levels, which is responsible for pathophysiological and neurotoxic malfunctions (Rodrigo & Felipo, 2006). The functional foods including naturally occurring bioactive substances and foods supplemented with bioactives (probiotics and antioxidants) could afford hepatoprotective effects (Al-Sheraji et al., 2013) and the present study validates the preventive action of fisetin on hyperammonemia.

In addition, dysfunctional or abnormal circadian rhythms have been associated with metabolic syndrome and liver diseases. Investigations on circadian rhythms in normal and pathological states can be employed to improve the perceptive of pathological processes and therapeutic approaches to diseases (Zelinski, Deibel, & McDonald, 2014). The circadian rhythm of blood corticosterone is modified in hyperammonemic rats (Llansola et al., 2013). Locomotor activity is interconnected to neuronal action and plasticity, which is recurrently impaired in hyperammonemia induced hepatic encephalopathy (HE) (Apelqvist, Hindfelt, Andersson, & Bengtsson, 1997; Felipo & Butterworth, 2002). Patients with HE showed impairment of the sleep–wake or rest–activity cycle (Wilkinson, Smeeton, & Watt, 2010).

Ammonia toxicity leads to astrocyte swelling, dysfunction and brain edema (Norenberg et al., 1991; Ott, Clemmesen, & Larsen, 2005). Astrocyte swelling stimulates signal transduction processes and hinders cellular metabolism. A deregulation of cell volume homeostasis due to increased glutamine in brain is a main pathogenic event of ammonia toxicity and HE (Zhok & Norenberg, 2000). Astrocytic swelling, further, may cause brainstem herniation and death (Clemmenson, Larsen, Kondrup, Hansen, & Ott, 1999).

In addition, hyperammonemia deregulates the glutamate–NO–cGMP pathway in brain neurons of rats, resulting in reduced synthesis of cGMP owing to suppression of soluble guanylate cyclase (sGC) (Hermenegildo et al., 1998; Monfort, Munoz, Ayadi, Kosenko, & Felipo, 2002). Inhibition of sGC by nitric oxide (NO) and thereby the glutamate–NO–cGMP pathway could be accountable for the neurological alterations initiated in HE (Rodrigo et al., 2010). Glial fibrillary acidic protein (GFAP) is mainly expressed in astrocytes and constitutes a selective marker for normal and pathological conditions and is also involved in the regulation of cell volume (Belanger, Desjardins, Chatauret, & Butterworth, 2002; Eng, Ghirnikar, & Lee, 2000). The specific expression of GFAP arbitrates shape, structure and motility of astrocytes (Eng et al., 2000). The loss of GFAP is also associated with astrocytic swelling (Belanger et al., 2002).

At present, the key disadvantages of numerous antihyperammonemic agents/therapies are the recurrence of symptoms and staid undesirable effects after their discontinuation (Braissant, McLin, & Cudalbu, 2013). Consequently, development and monitoring of drugs averting hyperammonemia is indispensable. We conjectured that fisetin (a bioactive antioxidant) could be valuable in averting hyperammonemia, and evaluated the defensive effect of fisetin by assessing (a) the levels of ammonia, urea, uric acid, creatinine, liver markers, lipid peroxidation products and antioxidants in circulation, liver and brain, (b) circadian locomotor activity rhythms, and levels of glutamine and glutamate in brain and (c) mRNA expression of sGC and GFAP in brain.

2. Materials and methods

2.1. *Animals and maintenance*

Male rats (Wistar strain, body weight 190–210 g) obtained from the Animal House, Faculty of Medicine, Annamalai University were used for investigations. The rats were dwelled in polypropylene cages and were fed with standard diet pellets (Hindustan Lever Ltd., Bangalore, India) and drinking water *ad libitum*. Standard conditions of temperature (24 ± 2 °C) with light:darkness (12:12 h) cycles were maintained throughout the study. The study protocols were endorsed by the ethical committee (Approval No.: 737; dated 02/09/2010), Annamalai University (Reg. No. 160/1999), compliant with the guiding principles of Indian Council of Medical Research (ICMR), New Delhi, India.