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Chrysin, a flavonoid attenuates histological changes of hyperammonemic rats: A dose dependent study

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ABSTRACT

Chrysin (5,7-dihydroxyflavone) is a major component of some traditional medicinal herbs present in honey, propolis and many plant extracts. The study was aimed to illuminate the effect of chrysin in the pathogenesis of ammonium chloride (NH_4Cl) induced hyperammonemic rat model in a dose dependent manner. Rats were injected with NH_4Cl (100 mg/kg b.w.) by intraperitonially (i.p) thrice a week for 8 consecutive weeks for the induction of experimental hyperammonemia. Hyperammonemic rats were treated with chrysin by orally at a dose of 25, 50 & 100 mg/kg b.w. respectively. Protective effect of chrysin against hyperammonemia was evaluated by performing biochemical estimations and morphopathological investigations of hematoxylin and eosin stained sections of liver, brain and kidney tissues. Supplementation of chrysin reinstated the levels of blood ammonia, plasma urea, uric acid, total bilirubin, creatinine, brain glutamate, glutamine, nitric oxide (NO) and the activities of Na^+/K^+-ATPase, and liver marker enzymes. On the other hand increased level of plasma urea was observed in chrysin treated rats as compared with hyperammonemic rats. Chrysin administration caused distortion of hepatic, brain and kidney architecture as shown by histological examination. Chrysin at a dose (100 mg/kg b.w.) showed an utmost decline in the level of all biochemical estimations. Both biochemical and morphological studies clearly revealed that chrysin protects against cell injury induced by ammonia intoxication in a dose-response manner with respect to endogenous antioxidants and hypoammonemic effects.

1. Introduction

Urea cycle, an effective system converts waste nitrogen from protein into urea subsequently excreted from the body. Total or partial deficiency in urea cycle results in an accumulation of ammonia and other products. Ammonia detoxification is therefore of utmost significance for maintaining homeostasis, and it takes place mainly through the specific pathway, urea synthesis. Due to compromised urea cycle during the liver failure condition, blood ammonia level gets elevated causing hyperammonemia (HA) [1]. HA is a metabolic disorder associated with a variety of situations including congenital urea cycle disorders, distal renal tubular acidosis, reye's syndrome, acute fulminant hepatic failure and organic acidemias. Also, it can be caused by a variety of inherited or acquired disorders, the most common being urea cycle disorders with an overall prevalence estimated at 1:8200 in the United States [2]. It may exceed the current estimates (1:8000–1:44,000 births) [3].

At hyperammonemic conditions, ammonia diffuses from the blood into the brain tissue without any hindrance [4]. Consequently, the concentrations of ammonia in the brain tissue can rise as high as 5 Mm. Since the brain lacks an effective urea cycle, cerebral ammonia removal relies mainly on the formation of aminoacids, *i.e.*, of glutamine [5] by the astrocytic specific enzyme glutamine synthetase (GS). During acute hyperammonemia *in vivo* glutamine synthesis is increased as a means to detoxify excess ammonia. The prevailing hypothesis that an osmotic disturbance induced by the astrocytic accumulation of glutamine in these cells leads to astrocytic swelling and consequently brain edema [6]. Ammonia intoxication not only causes astrocyte swelling, it can also alter neurotransmission, mitochondrial function, and induces oxidative/nitrosative stress [7].

Na^+/K^+-ATPase, a membrane bound enzyme is ubiquitous in nature and mammalian central nervous system, predominantly

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found in glial and nerve terminals [8]. It plays an important role in the maintenance of membrane potentials. It is present at high concentrations in the brain cell membrane, consuming about 40–50% of the adenosine tri phosphate (ATP) generated in this tissue, and is highly responsive to changes in membrane fluidity [9,10]. Ammonium ions (NH_4^+) can affect the membrane potential of nerve cells by altering ionic shifts. These effects of NH_4^+ are attributable to its K^+ and Na^+ like behaviour [11].

NO, the end product of the enzyme Nitric oxide synthase (NOS) influences various physiological processes in all tissues. NO is recognized as a potent neuroprotective antioxidant, and a mediator of cerebral edema under pathological conditions [12,13]. The function and formation of NO are altered under acute and chronic neuropathological conditions. While NO normally functions as a physiological cerebral blood-flow modulator and a neuronal mediator, when it exceeds it is neurotoxic. Increased NO production was also shown to be associated with disorders without significant neuronal damage such as hepatic encephalopathy (HE) and hyperammonemic syndromes [14,15]. Excess ammonia induces NOS, which leads to increased production of NO and other toxic free radicals as well as thiobarbituric acid reactive substances in brain which leads to oxidative stress and tissue damage [16].

Free radical induced damage in oxidative stress has been confirmed as a contributor to the pathogenesis and pathophysiology of many chronic health problems such as various neurodegenerative diseases [17]. Pathogenesis of hyperammonemia is interrelated with free radical induced oxidative/nitrosative damage [18]. Ammonia induces oxidative and nitrosative stress may results enhanced reactive oxygen species/reactive nitrosative species (ROS/RNS) generation or from a decay of the antioxidant protective ability, being characterized by the lower capacity of endogenous systems to fight against the oxidative attack directed towards target biomolecules [19]. Ammonia toxicity results in lipid peroxidation and free radical generation causes hepatic failure and dysfunction, which is a primary cause of neurological disorders and alterations in the function of the central nervous system (CNS), associated with hyperammonemia [20]. Therefore, we have tried to find appropriate multipotent agents with both antioxidant and free radical scavenging properties from the phytomedicine against hyperammonemia without any side effects.

Recently, there is growing interest in elucidating the biological roles of plant secondary metabolites against various human diseases. Several epidemiological studies suggest that supplementation of flavonoids rich diet prevents various diseases, especially the neurodegenerative diseases [21]. Chrysin (5, 7-dihydroxyflavone), a naturally wide distributed flavonoid, which is found in honey, bee propolis and various plants [22] (Fig. 1a). Chrysin has been reported to have numerous beneficial pharmacological activities, such as antidiabetogenic, antioxidant, anticancer, antiestrogenic, antihypertensive, antiinflammatory, antiviral and anxiolytic activities [22]. In this context, the present study was undertaken to investigate the neuroprotective effect of chrysin on the levels of blood ammonia, plasma urea, uric acid, creatinine, total bilirubin brain glutamate, glutamine, NO, activities of Na^+/K^+-ATPase, liver marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and the analysis of histological changes in the liver, brain and kidney tissues.

2. Materials and methods

2.1. Chemicals

Chrysin was purchased from Sigma-Aldrich Chemical Company, India. It was dissolved in corn oil and freshly prepared prior to each administration. All other chemicals were of analytical grade and were obtained from Himedia.

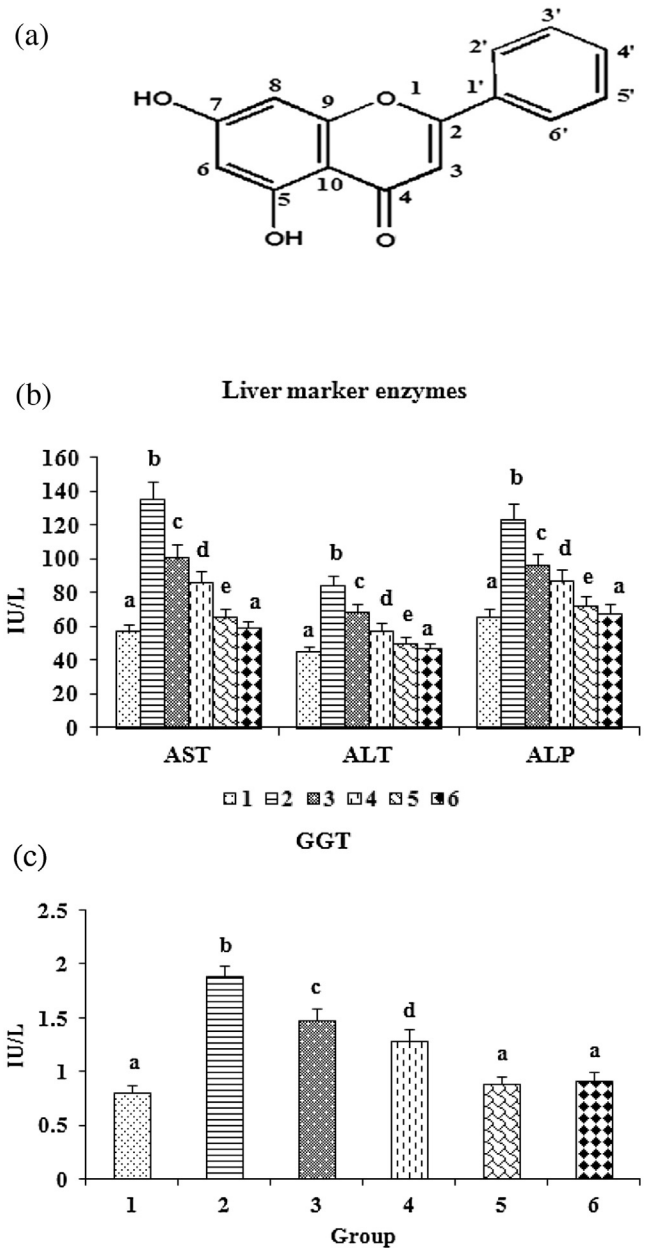

Fig. 1. a. Structure of chrysin, b & c. Activities of liver marker enzymes in normal and experimental rats.
1. Normal; 2. NH_4Cl(100 mg/kg b.w.); 3. NH_4Cl + chrysin(25 mg/kg b.w.); 4. NH_4Cl + chrysin (50 mg/kg b.w.); 5. NH_4Cl + chrysin(100 mg/kg b.w.); 6. Chrysin (100 mg/kg b.w.);
Values are given as mean ± S.D from each group (n = 6). Values not sharing a common superscript alphabet vary significantly at $p < 0.05$ (DMRT).

2.2. Experimental animals

Adult male albino Wistar rats, weighing 180–200 g reared in the Central Animal House, Rajah Muthiah Medical College, Annamalai University were used for the studies. The rats were housed in polypropylene cages lined with husk, renewed every 24 h, and provided with food and water *ad libitum*. They were maintained in a normal environment under standard conditions of temperature (22 ± 2 °C) and humidity (45–64%) with alternating light/dark (LD 12:12) cycle. The rats were fed with standard pellet diet. The