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Original article

In vivo restoration of hepatic and nephro protective potential of hesperidin and ellagic acid against mercuric chloride intoxicated rats

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ABSTRACT

The present experimental study is to investigate the efficacy of some phytochemicals on heavy metal intoxicated animals. Commonly phytochemicals have played a vital role for protective effect of oxidative stress, which is induced by heavymetals in animals. At sub-lethal dose of mercuric chloride (1.23 mg/kg body weight) treated rat liver tissue shows hepatic cell damage and alteration of its metabolic activities by the way of liver marker enzymes. The hepato-protective effect of Hesperidin and ellagic acid was tested against mercuric chloride induced hepato-toxicity in rats. In the present study, drastically altered in the level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Lactic dehyrogenase (LDH), bilirubin, albumin, cholesterol, urea and creatinine levels were observed in the blood serum of mercury intoxicated rats. The activity of liver marker enzymes such as ALT AST, ALP and LDH were significantly increased and albumin was simultaneously decreased in mercuric chloride intoxicated rats. Administration of Hesperidin and Ellagic acid (5 mg/kg body weight) on mercuric chloride intoxicated rats not only reduced the liver markers enzymes and bilirubin and cholesterol levels and also maintain their level to near normal condition. Hesperidin and ellagic acid alone treated animals did not alter the ALT, AST, ALP, LDH, bilirubin, albumin, cholesterol urea and creatinine levels in serum. Our results indicate that treatment for hesperidin and ellagic acid exhibited the strong hepatoprotective activity against mercuric chloride induced hepatotoxicity.

1. Introduction

Heavy metals exert many toxic effects on living systems. The molecular mechanisms underlying their toxicity are not well understood. Hg and its compounds are causing biochemical alterations in various tissues through diverse mechanisms such as lipid peroxidation, formation of reactive oxygen species [1], altering protein synthesis via binding to thiol groups [2]. Mercury inhibits/altered the number of enzymes activity and provokes cell damage. Mercury has high affinity to lipid allowing movement across cell membranes and can interfere with cell metabolism [3]. The principle toxic effects of mercury mainly involve the formation of complexes with thiol groups, which may lead to formation and excess production of oxidative stress in animal. Such type of oxidative stress turns to severe the inbuilt mechanism of body fails to alleviate the cell damage. Jagadeesan and Sankarsamipillai [4] have demonstrated that mercury decreases the anti-oxidative systems and produces oxidative damages via H_2O_2 generation thereby leading to lipid peroxidation. Mercuric chloride is highly toxic and corrosive. Once absorbed into blood stream, inorganic mercury combines with proteins in the plasma or enters the red blood cells.

The liver is a major site of metabolism for mercury and its accumulates in the kidneys, hence resulting in severe damage [5]. It increases the production of many endogenous oxidants, such as hydrogen peroxide [6], depletes protective antioxidants such as glutathione and reduces free radical scavenging systems. Liver is an important site for the synthesis of many serum proteins and the level of serum proteins are decreased in hepatic diseases. The oxidative damage of some amino acid is considered as the major cause of metabolic dysfunction in hepatic damage [7].

The toxic effects of divalent mercury can be prevented to some extent either by chelating or enhancing antioxidant defense mechanisms [8,9]. The ability of polyphenols to protect cell from oxidative stress has been demonstrated. However, polyphenol compounds could have both antioxidant and prooxidant properties, depending on the concentration and free radical source [10,11]. Generally, phenolic compounds effectively scavenge free radicals and inhibit NO production, independent of their antioxidant properties. ROS production is a naturally occurring process. A variety of enzymatic and non-enzymatic antioxidants are involved to protect cell against ROS.

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Hesperidin, a natural antioxidant, has been reported to possess various pharmacological activities. It is commonly used in traditional medicines as a combination product under the trade name hesperidin is a flavanone glycoside abundantly found in sweet orange and lemon and is an inexpensive byproduct of citrus cultivation [12–14]. Supplementation of hesperidin also helps in reducing edema or excess swelling in the legs due to fluid accumulation. Many researchers have examined the antioxidant activity and radical scavenging properties of hesperidin using a variety of assay system [15,16]. Hesperidin has been shown to have antioxidant activity against the cellular oxidative stress associated with neurodegenerative diseases. This flavonoid also attenuated decreases of glutathione peroxidase and glutathione reductase activity and decreased DNA damage in H_2O_2-induced PC12 cells [12], and also inhibited low-density lipoprotein oxidation. Oral administration of hesperidin has protective effects against gamma radiation-induced hepatocellular damage and oxidative stress in rats [12,17]. The pharmacokinetic parameters of hesperidin were measured for total hesperidin in healthy. Further, hesperidin offers protection to cultured human peripheral blood lymphocytes against radiation induced cellular damage [18].

Ellagic acid exhibited anticarcinogenic potential [19,20]. Ellagic acid has been shown to exert a potent scavenging action on super oxide anion and hydroxyl anion in vitro, as well as the protective effect against lipid peroxidation [21]. The antioxidant mechanism of ellagic acid may include the following interventions: scavenging of O_2, OH, peroxy radical and peroxynitrite [22,23]. Ellagic acid prevents the formation of various tumors, this mechanism of action can be possible because compounds such as ellagic acid explicitly interact with the cells walls or sites with facility to complex proteins, preventing the proliferation of metastatic cells [24].

The aim of the present experimental work was to prove the possibility to use the bioactive compounds of hesperidin and ellagic acids against mercuric chloride induced hepatotoxicity.

2. Materials and methods

2.1. Chemicals

Mercuric chloride ($HgCl_2$), Hesperidin, Ellagic acid and all other necessary reagents of analytical grade were bought from Hi-Media laboratories Ltd, Mumbai, India.

2.2. Animals

Healthy male albino rats (150–200 g) were obtained from the Central Animal House, Department of Experimental Medicin, Raja Muthiah Medical College and Hospital Annamalai University and maintained in an air condition room ($25 \pm 3\,^{\circ}C$) with a 12-h light/12-h dark cycle. Feed and water were provided ad libitum to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No: 160/1999/CPCSEA, Proposal Number: 954), Annamalai University, Annamalainagar.

2.3. Experimental design

The animals were randomized and divided into six groups, each group containing six rats. The toxic dosage of mercuric chloride has been determined from our previous study as sufficient to elicit mild or moderate oxidative stress for mercuric chloride [4].

- Group I: only vehicle (0.9% NaCl) was given to these animals (Control).
- Group II: the animals were administered $HgCl_2$ 1.23 mg/kg body weight in 0.9% NaCl intraperitoneally for 7 days.
- Group III: the animals were administered orally Hesperidin (5 mg/kg body weight) alone for 7 days.
- Group IV: the animals were administered Hesperidin after the intoxication of mercuric chloride administration.
- Group V: the animals were administered orally Ellagic acid (5 mg/kg body weight) alone for 7 days.
- Group VI: the animals were administered Ellagic acid after the intoxication of mercuric chloride administration.

2.4. Sample preparation

At the end of the experimental duration, rats were fasted overnight and anaesthetized with intramuscular injection of ketamine hydrochloride (24 mg/kg body weight) and sacrificed by cervical dislocation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 min. Plasma was separated by collecting blood in tubes containing heparin and centrifuged at 2000 rpm for 10 min. The serum samples were used for hepatic marker assay ALT AST, ALP, LDH, bilirubin, albumin and chloestrol, nephritic marker assay urea and Creatinine.

2.5. Biochemical analysis

The alkaline phosphate was estimated by King and Armstrong method [25]. The activity of AST and ALT was determined by adopting the method of King [26]. The activity of lactate dehydrogenase was assayed by the method of King [27]. Albumins in the serum were estimated by Biuret method (Reinhold), [28].The level of serum Bilirubin was estimated by the method of Mallay and Evelyn [29]. Total cholesterol in the plasma and tissues was estimated by the enzymatic method described by Allain et al. [30]. Serum urea was estimated by using the diagnostic kit based on the method of Fawcett and Scott [31]. Serum Creatinine was estimated by the method of Bonsnes and Taussly [32].

2.6. Statistical analysis

Values are given as mean ± S.D. for six rats in each group. The data for various biochemical parameters were analyzed using analysis of *t*-test and the group means was compared by Duncan's multiple range test (DMRT) [33]. Values were considered statistically significant when $P < 0.05$ and the values sharing a common superscript did not differ significantly.

3. Results

Table 1 shows that the level of ALT AST, ALP, LDH and bilirubin was significantly increased in mercury intoxicated rat blood serum compared to control. On administration of hesperidin and ellagic acid to animals, the level of ALT AST, ALP, LDH and bilirubin was significantly decreased near to normal level compared to mercuric chloride induced rat.

Table 2 shows that level of cholesterol, urea and Creatinine was significantly increased in mercuric chloride induced rat blood serum compared to control group. The hesperidin and ellagic acid treated rats were cholesterol, urea and Creatinine significantly restored near normal level compared to mercuric chloride intoxicated rats. Albumin activity is a significant decreased in mercuric chloride intoxication rats. Treatment of hesperidin and ellagic acid orally administrated the rats the albumin activity was significantly increased near to normal level compared to mercuric chloride intoxicated rat blood serum.