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Original article

Hepato-ameliorative effect of hesperidin and ellagic acid on mercuric chloride intoxicated rats

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ABSTRACT

Protective efficacy of Hesperidin and Ellagic acid on hepatotoxicity induced by mercuric chloride for 7 days in rats, *Rattus norvegicus*, were carried out in the present study. At sub-lethal dose of mercuric chloride (1.23 mg/kg body weight) was administrated in rats for 7 days through oral dose. After completing the scheduled exposure time, the intoxicated rats were sacrificed and then whole liver organ was isolated immediately and they used for bio-enzymological analyses. In the present experimental study, the following biochemical and bio-enzymological studies were carried out to find the levels of lipid peroxidation (LPO) and reduced glutathione (GSH) and super oxidedismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X) activities in whole liver tissue. The results revealed that treatment with mercuric chloride caused marked elevation in the level of free radicals (LPO) and simultaneously decreased in the level of SOD, CAT, GP_X, activities and GSH content in rat liver tissue. The hesperidin and ellagic acid (5 mg/kg body weight) treatment on mercury-intoxicated rats were restoring these oxidant and antioxidant activities near to normal level when compared to mercuric chloride intoxicated groups. These results suggested that the protective efficacy of hesperidin and ellagic acid on mercuric chloride induced hepatotoxicity in rats have been proven.

1. Introduction

Free radical production and oxidative stress is one of the mechanisms of tissue damage in heavy-metal toxicity in animals. Mercuric chloride is one of the heavy-metal, which is present in an inorganic mercury compound with ionic mercury. Many inorganic compounds of mercury have used in various products and agriculture medicines etc. Intoxication with mercury and its compounds occurs through environmental occupational or accidental exposure [1,2]. Mechanisms of tissue damage have been caused by mercury and its compounds are not completely clarified. But many studies confirm that the promotion of oxidative stress as important factor in tissues damage. Mercury and its compounds are transition metal and it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides [3] in animal when it was exposed. The ROS enhances the subsequent iron of lipid peroxides and the highly reactive hydroxyl radical. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell [4].

Mercury also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase and glutathione peroxidase [1]. Mercury induced oxidative stress make an important contribution to molecular mechanism for liver injury [5,6]. Recent evidence also shows that it may cause severe oxidative damage, thus mercury is proved to be a potential oxidant in the category of environmental factors [7]. Many authors suggested that mercury ions compromise both the antioxidant potential of GSH and promote formation of reactive species via thiol complex [8–10]. Antioxidant defense systems protect cellular homeostasis from oxidative disruption by reactive molecules generated through the reduction of molecular oxygen. The efficient functionality of these mechanisms requires the concerted action of the individual systems [11].

Hesperidin is one of the most abundant natural flavonoids, which is present in a large number of fruits and vegetables [12]. Hesperidin (3',5,7-trihydroxy-4'-methoxy-flavanone-7-rhamnoglucoside) is mainly isolated from citrus fruits. A number of pharmacological properties of hesperidin have been reported. For example hesperidin exhibited antihypercholesterolemic activity [13,14], antihypertensive and diuretic effects [15,16], etc. Hesperidin have many biological effects including anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant effects and neuroprotective actions [16]. It exhibits anti-oxidative properties by several different mechanisms, such as scavenging of free radicals, chelation of metal ions such as iron and copper which are of major importance for the initiation of radical reactions, inhibition of enzymes responsible for free radical generation and facilitation endogenous

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antioxidative defense system [16,17]. The antioxidant properties of flavonoids depend on both metal-chelating properties and free radical scavenging of ROS [16].

Ellagic acid (2,3,7,8-Tetrahydroxy-chromeno[5,4,3,-cde]chromene-5,10-dione) is a polyphenol antioxidant and it also found in numerous fruits and vegetables. The highest levels of ellagic acid are found in raspberries [18]. Ellagic acid has been shown to exert a potent scavenging action on super oxide anion and hydroxyl anion. And it also acts as great protective effect against lipid peroxidation. Ellagic acid is pharmacologically active and has been found to control hemorrhage in animals and in humans, presumably as a result of its ability to activate Hageman factor [19]. Number of experimental works suggests that red raspberry may reduce the levels of glucose (blood sugar) in animals [20,21]. The exact molecular mechanism of ellagic acid is not completely understood. Hence, it could be suggested that the leakage of enzymes from the hepatocellular membrane is decreased with administration of ellagic acid was established [22].

The aim of the present examination was to evaluate the antioxidant activity and hepatoprotective effect of hesperidin and ellagic acid on mercuric chloride induced hepatic cell damage in rats.

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 procured 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 (sub-lethal dose of $HgCl_2$ 1.23 mg/kg body weight) from our previous study carried out in our lab. And it have sufficient to elicit mild or moderate oxidative stress in rats (Sankarsamipillai and Jagadeesan, 2007):

Group I ($n = 6$): only vehicle (0.9% Nacl) was given to these animals (Control);
Group II ($n = 6$): the animals were administered $HgCl_2$ 1.23 mg/kg body weight in 0.9% Nacl intraperitoneally for 7 days;
Group III ($n = 6$): the animals were administered orally Hesperidin (5 mg/kg body weight) alone for 7 days;
Group IV ($n = 6$): the animals were administered Hesperidin after the intoxication of mercuric chloride administration;
Group V ($n = 6$): the animals were administered orally Ellagic acid (5 mg/kg body weight) alone for 7 days;
Group VI ($n = 6$): the animals were administered Ellagic acid after the intoxication of mercuric chloride administration.

At the end of the experimental duration, the rats were anaesthetized with intramuscular injection of ketamine hydrochloride (24 mg/kg body weight) and sacrificed by cervical dislocation. The whole kidney tissue was isolated immediately from the animal and kept in an ice-cold saline and then used for estimation of oxidant and antioxidant properties and also for histological studies.

2.4. Estimation of lipid peroxidation (TBARS)

The concentration of TBARS in the liver tissue was estimated by adopting the method of Nichans and Samuelsen [23]. Known amount of whole liver tissue homogenate was prepared in Tris-HCl buffer (pH 7.5). 1 ml of the tissue homogenate was taken in a clean test tube and 2.0 ml of TBA-TCA-HCL reagent was added and then mixed thoroughly. The mixture was kept in a boiling water bath ($60\,^{\circ}C$) for 15 min. After cooling, the mixture was taken to read the absorbance of the chromophore at 535 nm against the reagent blank in a UV-visible spectrophotometer (Spectronic-20, Bausch and Lamb). About 1, 1', 3, 3' tetra methoxy propane was used to construct the standard graph. Values were expressed as *n*-moles of MDA released per 100 mg.

2.5. Estimation reduced glutathione (GSH) activity

The level of reduced glutathione in liver tissue was estimated by the method of Beutler and Kelley. A known weight of tissue was homogenized in phosphate buffer (0.1 M. pH 7.0) and centrifuged at 2500 rpm for 5 min. About 0.2 ml of the sample (Supernatant) was taken in a clean test tube and 1.8 ml of EDTA solution was added. To this 3.0 ml of precipitating reagent was added and mixed thoroughly and kept for 5 min before centrifugation at 3000 rpm for 10 min. In a clean test tube, 2.0 ml of the content mixture was taken and to this 4.0 ml of 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagents were added. The appearance of yellow colour was read at 412 nm in UV-visible spectrophotometer (Spectronic-20, Bausch and Lamb). A set of standard solution containing 20–100 μg of reduced glutathione was treated similarly. Values are expressed as μg per 100 mg protein.

2.6. Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase in the liver tissue was assayed by adopting the method of Kakkar [25]. The liver tissue was homogenized with 2.0 ml of o.25 M sucrose solution and the centrifuged the contents at 10,000 rpm for 30 min in a cold centrifuge. After completing the centrifugation the supernatant was taken in a clean test tube and the content was dialysed against the Tris-HCl buffer and then mixed the contents thoroughly. The contents were centrifuged again at 3000 rpm for 15 min. The supernatant was taken in a clean test tube and then 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of phenazine methosulphate and 0.3 ml of nitroblue tetrazolium reagents were added. The sample mixture (enzyme preparation) was kept in water bath at $30\,^{\circ}C$ for 90 s and appropriately diluted enzyme preparation in a total volume of 3 ml with double distilled water. The reaction was started by the addition of 0.2 ml NADH. After completing the incubation period, the reaction was stopped by the addition of 1m1 glaicial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 min and then centrifuged the contents at 3000 rpm for 5 min and n-butanol layer was separated the colour density of the chromogen in n-butanol was measured in an UV spectrophotometer at 510 nm. A system devoid of enzyme served as control. The enzyme concentration required to inhibit the chromogen produced by 50% in 1 min under standard conditions was taken as one unit. The specific activity of the enzyme was expressed as unit/min/mg of protein for tissues.