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Original research article

Hesperidin alleviates cisplatin-induced hepatotoxicity in rats without inhibiting its antitumor activity

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ABSTRACT

Background: Hesperidin, a naturally occurring flavonoid, exerts many clinically appreciable effects such as anti-oxidant, anti-allergic and anti-inflammatory actions. The present study aimed to investigate the possible protective effects of multiple doses of hesperidin against cisplatin-induced acute hepatotoxicity in rats.

Methods: Hesperidin (100 or 200 mg/kg *po*) was given to rats one day before cisplatin (7.5 mg/kg, *ip*) injection. All animals were sacrificed 5 days after cisplatin injection and blood samples were collected for determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, triglycerides (TG) and total cholesterol levels. Liver samples were used for the determination of malondialdehyde (MDA), glutathione (GSH), total nitrate and nitrite contents. Western blot analysis was used for the assessment of NF-κB and p-Akt expression and histopathological examination was also performed.

Results: Results showed that hesperidin significantly reduced cisplatin-induced elevations in serum ALT and AST activities, TG and total cholesterol levels. It also reduced cisplatin-induced oxidative stress by significant reduction in liver MDA and NO content and elevation of GSH content. In addition, hesperidin significantly counteracted cisplatin-induced increased NF-κB expression and decreased p-Akt expression. Histopathological examination revealed that hesperidin greatly protected liver against cisplatin-induced injury. Moreover hesperidin did not inhibit the cytotoxic effect of cisplatin on cancer cells as determined by MTT assay.

Conclusion: Hesperidin decreased cisplatin-induced functional and histopathological liver damage in a dose-dependent manner without affecting its potential cytotoxic effect.

Introduction

Cisplatin, one of the most potent chemotherapeutic agents is used in the treatment of a variety of tumors [1]. Despite its beneficial antineoplastic effects, cisplatin causes many undesirable side effects on different tissues including nephrotoxicity, hepatotoxicity, cardiotoxicity and ototoxicity [2]. These side effects limit its application in clinical oncology as a powerful chemotherapeutic agent [3].

Nephrotoxicity and hepatotoxicity are dose limiting side effects in cisplatin-based chemotherapy [4]. Cisplatin accumulates in liver at significant amounts second to kidney [5]. Although cisplatin nephrotoxicity has been well studied, its hepatotoxicity still needs to be investigated.

Several studies cleared that the generation of reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical are involved in the mechanism of cisplatin toxicity [3] which leads to an elevation in lipid peroxidation (LPO), reduction in the level of protein bound sulfhydryl groups and glutathione [6].

So, it deemed of great importance to investigate ways for preventing the dose-limiting side effects of cisplatin at its tumoricidal doses for safer clinical use. Several studies have proved that the use of

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different anti-oxidants and anti-inflammatory agents is effective against cisplatin deleterious effects on kidney and liver [7].

Great attention has been given to dietary and natural products-derived anti-oxidants and their protective effect against cisplatin-induced toxicity [8]. Hesperidin, a naturally occurring flavonoid found abundantly in vegetables and fruits [9]. It is an inexpensive byproduct of citrus cultivation and is the major bioflavonoid in the sweet orange and lemon [10]. Hesperidin exerts many beneficial effects such as anti-oxidant, anti-allergic and anti-inflammatory actions [11]. It has also anti-carcinogenic effects in tongue, esophagus, colon and urinary bladder in rat models of carcinogenesis [12].

The aim of the present study was to investigate the possible protective effects of multiple doses of hesperidin against cisplatin-induced hepatotoxicity in rats which in turn could increase cisplatin activity and decrease its toxicity to normal tissues. In addition, the possible mechanisms of hepatotoxicity induced by cisplatin administration and the possible effect of hesperidin on the anticancer activity of cisplatin were investigated.

Materials and methods

Animals

Adult male Wistar rats (120–150 g) obtained from the National Research Centre (Giza, Egypt) were used in the current study. Animals were housed in 10 cages, 6 per cage with free access to water and food and maintained at 25 ± 2 °C, under a 12/12 h light–dark cycle. Animal handling in this study was carried out according to guidelines of the Ethics Committee of Faculty of Pharmacy, Cairo University.

Drugs and chemicals

Cisplatin, silymarin, hesperidin, thiobarbituric acid, Ellman's reagent, vanadium trichloride, N-(1-Naphtyl) ethylenediamine dihydrochloride, sulfanilamide and methylthiazole tetrazolium (MTT) were purchased from Sigma-Aldrich (USA). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) colorimetric assay kits were purchased from Randox, (UK). Triglycerides (TG) and total cholesterol (TC) commercial kits were purchased from Spinreact (Spain).

Antibodies against phospho-Akt [p-Akt (Ser473)] and NF-κB were purchased from Cell Signaling Technology (USA). Antibody against β-actin was purchased from Neomarkers (USA), and horseradish peroxidase (HRP)-conjugated secondary antibodies from Roche (USA). Chemiluminescence substrate kit was obtained from Pierce (USA). DC Bio-Rad protein quantitation reagents were from Bio-Rad (USA). Dulbecco's Modified Eagle's Medium (DMEM), penicillin, streptomycin and fetal bovine serum were purchased from Gibco (USA).

Experimental design

Fifty six rats were randomly assigned into seven groups (8 rats per group). Group I (Control gp): normal rats which received vehicle (2% Tween 80 *po*) for 7 days and a single *ip* injection of isotonic saline on the following day. Group II (Silymarin Control gp): rats received silymarin (100 mg/kg/day *po*) in 2% tween 80 for 7 days and a single *ip* injection of isotonic saline on the following day. Group III (Hesperidin Control gp): rats received hesperidin (200 mg/kg/day *po*) in 2% tween 80 for 7 days and a single *ip* injection of isotonic saline on the following day. Group IV (Cisplatin gp): rats received vehicle (2% tween 80, *po*) for 7 days and a single dose of cisplatin (7.5 mg/kg *ip*) on the following day [13]. Group V (Silymarin-treated gp): rats received silymarin (100 mg/kg/day *po*) in 2% tween 80 for 7 days and a single dose of cisplatin (7.5 mg/kg *ip*) on the following day, 1 h after silymarin administration [14]. Group VI (Hesperidin 100-treated gp): rats received hesperidin (100 mg/kg/day *po*) in 2% tween 80 for 7 days and a single dose of cisplatin (7.5 mg/kg *ip*) on the following day 1 h after hesperidin administration [15]. Group VII (Hesperidin 200-treated gp): received hesperidin (200 mg/kg/day *po*) in 2% tween 80 for 7 days and a single dose of cisplatin (7.5 mg/kg *ip*) on the following day 1 h after hesperidin administration [15].

At the end of the experiment, rats were fasted overnight, then euthanized and blood samples were collected. Serum was separated by centrifugation at 3000 r.p.m. for 10 min and used for various biochemical tests. For liver tissue biomarkers and histopathological estimations animals were sacrificed then liver was dissected out, washed with cold normal saline and dried between filter papers and either fixed in 10% formalin in saline for histopathological examination, homogenized for subsequent measurement of liver reduced glutathione (GSH), lipid peroxides and total nitrate and nitrite contents or stored at −80 °C for western blot analysis.

Determination of liver function

The enzymatic activity of ALT and AST were measured spectrophotometerically at 546 nm using a test reagent kit. TG and TC were measured to evaluate liver function using commercial assay kits at 505 nm.

Preparation of liver homogenate

A portion of each liver tissue was homogenized with 4 volumes of cold saline using a homogenizer to prepare 25% homogenate. The homogenates were centrifuged at 1000 × *g* for 15 min and the obtained supernatants were used for the estimation of liver biomarkers.

Measurement of liver lipid peroxides content

Lipid peroxidation, as malondialdehyde (MDA), was measured with the method of Thiobarbituric acid reaction according to the method described before [16,17]. **To** 0.25 ml of tissue homogenate, 1.5 ml of o-phosphoric acid (1%) and 0.5 ml of thiobarbituric acid (0.6%, w/v) were added. The mixture was heated in a boiling water bath for 45 min. After cooling, 2 ml of n-butanol were added and mixed vigorously to extract the pink color product followed by centrifugation at 3000 rpm for 15 min. The absorbance of the developed pink color in the n-butanol layer was measured at 520 nm using a double beam spectrophotometer. The values of MDA were expressed as *nmol/gm wet tissue*.

Measurement of liver GSH content

GSH was estimated according to the method described before [18,19] and expressed as *mg/gm wet tissue*. Protein was precipitated by adding 0.5 ml of the 5% sulfosalycilic acid to 0.25 ml homogenate, mixed and allowed to stand for 20 min. This was followed by centrifugation at 1000 rpm for 15 min at room temperature. Accurately, 0.25 ml of the resulting clear supernatant was taken and mixed with 1 ml phosphate buffer and 125 μl Ellman's reagent. The absorbance of the resulting yellow color was measured spectrophotometrically within 5 min at 412 nm. The blank of the sample was treated exactly as the sample but using bidistilled water.

Measurement of liver total nitrate and nitrite content

Total nitrate/nitrite (NOx) content was measured according to the method described by Miranda and others [20]. Cold absolute