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Hepatoprotective effect of grape seed proanthocyanidins on Cadmium-induced hepatic injury in rats: Possible involvement of mitochondrial dysfunction, inflammation and apoptosis

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ABSTRACT

The present study was undertaken to evaluate the possible ameliorative role of grape seed proanthocyanidins (GSP) against Cadmium (Cd) induced hepatic inflammation, apoptosis and hepatic mitochondrial toxicity in rats. Male Wistar rats were distributed in four experimental groups: control, GSP, Cd and Cd + GSP. Exposure to a hepatotoxic dose of Cd (5 mg/kg BW) caused liver damage, coupled with enhanced reactive oxygen species (ROS) generation, increased inflammation and apoptosis in liver with increased DNA damage in hepatocytes of rats. Mitochondria were isolated from the hepatic tissues of rats from each group. Our results showed significant decrease in the tri-carboxylic acid cycle enzymes, increased mitochondrial swelling, inhibition of cytochrome c oxidase activity and complex I–III, II–III and IV mediated electron transfer, decreased mitochondrial ATPases, a reduction in calcium content and mitochondrial oxygen consumption in Cd treated rats. All these molecular changes caused by Cd were alleviated by the pre-supplementation with GSP (100 mg/kg BW). The ultra structural changes in the liver also support our findings. From our results, it is clearly indicated that the free radical scavenging, metal chelating and antioxidant potentials of GSP might be the possible reason, responsible for the rescue action against Cd induced mitochondrial damage in the liver of rats.

1. Introduction

Cadmium (Cd) is one of the most toxic metals released into the environment and is known to be a hepatotoxic facet that can be transferred between various levels of the food chain. Cd belongs to the group of transition elements and adopts almost only one oxidation state +2 [1]. The toxic action of the Cd is recognized to be multifactorial. Cd acts as a catalyst in the oxidative reactions of biological micro molecules and therefore toxicities associated with this metal might be due to the oxidative tissue damage. Cd increases the production of reactive oxygen species (ROS) not through the Fenton like reaction [1], but the mechanism involves the interference with the activities of antioxidant, pro-oxidant and some other enzymes, alteration in thiol proteins, inhibition of energy metabolism and alteration in DNA structure and inhibits the activity of several crucial enzymes of the antioxidative defense system. Binding of Cd to sulphydryl group results in the primary injury of cells in mitochondria and secondary injury initiated by the activation of Kupffer cells have been mentioned as a possible mechanism of Cd induced toxic effects [2]. Activated Kupffer cells release proinflammatory cytokines and chemokines, which stimulate the migration and accumulation of neutrophils and monocytes in the liver, which amplificate the Cd, induced primary injury in hepatocytes [3]. The liver is one of the major target organs of both chronic and acute Cd exposure. While hepatocytes and endothelial cells of the liver sinusoids are supposed to be the primary cellular targets in liver [4].

Mitochondria are the key intracellular targets for different stresses, including Cd [5], but the mechanism of metal-induced mitochondrial damage is not fully understood. Mitochondria are the major source of ROS and thus a prime target of Cd induced hepatoxicity. About 1–4% of total consumed mitochondrial oxygen is incompletely reduced to the production of ROS [5]. Cd causes mitochondrial swelling, decreases the expression of genes that control mitochondrial activity, and finally, decreases mitochondrial oxidative capacity and ATP synthesis. Cd insult to mitochondria pertains to a structural damage as well as impairment in the activity of certain enzymes [6] modifies mitochondrial function and inhibits oxidative phosphorylation in the liver of rats.

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As oxidative stress is one of the key mechanisms of Cd-induced damages, it can be expected that the administration of some antioxidants should be an imperative therapeutic approach. Proanthocyanidins also called condensed tannins and are the oligomers of flavan-3-ol units. They are naturally occurring plant metabolites widely available in fruits, vegetables, nuts and barks and seeds. Grape seed constitutes the major proportion of proanthocyanidins. They are a class of phenolic compounds that takes the form of oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin, all of which contain water soluble molecules and a number of phenolic hydroxyls [7]. Current studies have shown that grape seed proanthocyanidin (GSP) can clear off free radicals, protect the over-oxidative damage caused by free radicals and prevent a range of diseases such as myocardial infarction, atherosclerosis, drug-induced liver and kidney injury [8]. In vivo studies have shown that GSP is a better free radical scavenger and inhibitor of oxidative tissue damage than vitamin C, vitamin E succinate, vitamin C and vitamin E succinate combined, and beta carotene [9]. One of the most advantageous features of proanthocyanidin free radical scavenging activity is that it is easily incorporated within the cell membranes [9]. The presence of both, hydrophobic and hydrophilic residues within the flavan-3-ol molecule, allows these compounds to interact with phospholipid head groups and be adsorbed onto the surface of membranes [10]. Several authors have studied the mitoprotective effect of some types of flavonoids, such as resveratrol [11] and quercetin [12]. These studies suggest that these natural compounds increase the aerobic capacity of muscle tissue and improve the mitochondrial function. Despite the prior research findings, very little is known about the protective effect of GSP against Cd induced mitochondrial oxidative stress in rats.

Previously, we have evaluated the parameters of oxidative stress in the course of Cd-induced hepatotoxicity, as well as the effect of GSP treatment on liver function, lipid peroxidation and activities of antioxidants in the liver of Cd intoxicated rats [10]. The results of our previous study serve as a step toward the development of a mechanism-based therapeutic approach against Cd induced mitochondrial toxicity in the liver of rats. Hence, provide the basis for the usefulness of GSP antioxidant therapy against Cd intoxication. The current study focused on the potential role of GSP against oxidative stress mediated mitochondrial toxicity and the therapeutic approach against Cd induced hepatic apoptosis, inflammation and DNA damage in the liver of rats.

2. Material and methods

2.1. Chemicals

GSP, containing approximately 54% dimeric, 13% trimeric procyanidins, and 7% tetrameric, proanthocyanidins were obtained from Jianfeng, Inc. (Lot No. G050412, Tianjin, China). $CdCl_2$ and other fine chemicals were obtained from Pfizer, India. Cytochrome c oxidase and Sigma diagnostics (I) Pvt., Ltd., Baroda, India, and Caspase-3 assay, all were obtained from the Sigma Aldrich Chemical Co. (St. Louis, MO). Reduced nicotinamide adenine dinucleotide (NADH) and sucrose were purchased from Himedia Laboratories Pvt., Ltd. (Mumbai, India). All other analytical grade chemicals were purchased from E. Merck (India).

2.2. Animals

Male Wistar rats weighing 170–190 g were used in this study for 28 days. They were maintained in an environmentally controlled animal house (temperature 24°–38 °C, Humidity 55 ± 10%) with a 12 h light/dark schedule and free access to deionized drinking water. The animal treatment and protocol employed were approved by the institutional Animal Ethics Committee, Annamalai university (Registration number: 1020/2013, CPCSEA).

2.3. Experimental design

In the present study, Cd was administered as $CdCl_2$ and administered intragastrically at a dose of 5 mg/kg body weight (BW)/day for 28 days. Twenty four male Wistar rats were divided into four groups consisting of six rats in each group:

Group I—served as a control group and received normal saline used as a vehicle, once in a day, for four weeks continuously.

Group II—rats were orally administered with GSP (100 mg/kg BW) in normal saline once a day for four consecutive weeks [10]

Group III—were orally administered with Cd as $CdCl_2$ (5 mg/kg BW) dissolved in saline once a day for four weeks [13]

Group IV—were orally pre-administrated with GSP (100 mg/kg BW) 90 min before the Cd (5 mg/kg.BW) once in a day for four weeks.

The animals from all the groups were provided with a pellet foodstuff from the Amrut laboratory animal feed, Pune, Maharashtra, India for feeding and water *ad libitum*. At the end of the experimental period, rats were fasted overnight and were anesthetized with pentobarbital sodium (35 mg/kg, IP) and euthanized by cervical decapitation. Blood was collected in tubes containing ethylene diamine tetraacetate (EDTA). The plasma was obtained after centrifugation (2000 × *g* for 20 min at 4 °C) and liver tissues were excised immediately and rinsed in ice-chilled physiological saline.

2.4. Cd concentration in the liver

Samples of the liver (about 100 mg) were weighed in Teflon PFA microwave digestion vessels previously washed in an ultrasonic bath for 30 min, rinsed in MilliQ water, left overnight in 10% ultrapure HNO_3, rinsed again with MilliQ water and left to dry at room temperature in a laminar flow hood. Samples were digested using 10 ml of HNO_3. The use of HNO_3 for digestion of biological samples is widely favored as it introduces less interference than other mineral acids in the analysis of biological samples by inductively coupled plasma mass spectrometry (ICP-MS) [14]. After cooling, the vessels were opened in a laminar flow box. The contents were transferred to acid cleaned polypropylene tubes and diluted to a volume of 10 ml with MilliQ water. All samples were diluted (1:4). For ICPMS, standards were prepared manually. Cd concentration was determined by the ICPMS method (ELAN-6000 model, PerkinElmer, Sciex, Toronto, Canada) according to the manufacturer's recommendation. The detection limit was 0.08 lg Cd/L. Daily controls ensured that the instrument satisfied the manufacturer's recommendations on performance criteria

2.5. Hepatic membrane-bound ATPases estimation

The sediment after centrifugation was resuspended in ice-cold Tris (hydroxymethyl) aminomethane (Tris)–hydrochloric acid (HCl) buffer (0.1 M) pH 7.4. This was used for the estimations of membrane-bound enzymes and protein content. The membrane-bound enzymes such as sodium ion (Na^+)/potassium ion (K^+)-ATPase, calcium ion (Ca^{2+})-ATPase and magnesium ion (Mg^{2+})-ATPase activities were assayed by estimating the amount of phosphorous liberated from the incubation mixture containing tissue homogenate, ATP, and the respective chloride salt of the electrolytes [15] [16]. Total protein content was estimated by the method described by Lowry et al. [17].