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Hepatoprotective effect of grape seed oil against carbon tetrachloride induced oxidative stress in liver of γ-irradiated rat

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ABSTRACT

Carbon tetrachloride (CCl_4) and ionizing radiation are well known environmental pollutants that generate free radicals and induce oxidative stress. The liver is the primary and major target organ responsible for the metabolism of drugs, toxic chemicals and affected by irradiation. This study investigated the effect of grape seed oil (GSO) on acute liver injury induced by carbon tetrachloride (CCl_4) in γ-irradiated rats (7 Gy). CCl_4-intoxicated rats exhibited an elevation of ALT, AST activities, IL-6 and TNF-α level in the serum. Further, the levels of MDA, NO, NF-κB and the gene expression of CYP2E1, iNOS and Caspase-3 were increased, and SOD, CAT, GSH-Px, GST activities and GSH content were decreased. Furthermore, silent information regulator protein 1 (SIRT1) gene expression was markedly down-regulated. Additionally, alterations of the trace elements; copper, manganese, zinc and DNA fragmentation was observed in the hepatic tissues of the intoxicated group. These effects were augmented in CCl_4-intoxicated-γ-irradiated rats. However, the administration of GSO ameliorated these parameters. GSO exhibit protective effects on CCl_4 induced acute liver injury in γ-irradiated rats that could be attributed to its potent antioxidant, anti-inflammatory and anti-apoptotic activities. The induction of the antioxidant enzymes activities, down-regulation of the CYP2E1, iNOS, Caspase-3 and NF-κB expression, up-regulation of the trace elements concentration levels and activation of SIRT1 gene expression are responsible for the improvement of the antioxidant and anti-inflammatory status in the hepatic tissues and could be claimed to be the hepatoprotective mechanism of GSO.

1. Introduction

Liver injuries are one of the most degenerative diseases worldwide and can lead to different complications. The liver is the primary and major target organ responsible for the metabolism of drugs and toxic chemicals. Further, the liver plays a central role in transforming and clearing the metabolites and xenobiotic, therefore, it is susceptible to the toxicity from these agents [1,2]. Carbon tetrachloride (CCl_4) and ionizing radiations are well known environmental pollutants that generate free radicals and induce the oxidative stress [3]. Liver injury induced by CCl_4 is the best-characterized system of the xenobiotic-induced free radical mediated hepatotoxicity and is a commonly used model for the screening of the anti-hepatotoxic and hepatoprotective activity of drugs and natural products [4]. The most prominent pathological characteristics of CCl_4-induced hepatotoxicity are fatty liver, cirrhosis and necrosis that have been resulted from the formation of reactive intermediates such as trichloromethyl (•CCl_3 and/or •CCl_3OO) and oxygen centered lipid radicals (LO• and/or LOO•). These free radicals are pivotal in CCl_4-induced hepatotoxicity, which are generated during CCl_4 metabolism by hepatic cellular cytochrome P450 [4,5]. In addition, the activation of Kupffer cells also contributes to the liver injury through releasing both direct toxic products and cytokines which promote the inflammatory response [6].

On the other hand, the widespread use of radiation in the diagnosis, industry and the energy sector and its accidental exposure due to nuclear accidents and nuclear terror attacks requires a safeguard against human exposures [7]. Radiation being increasingly used for medical and occupational purposes and it is an established weapon in the diagnosis and the therapy of cancer. The destructive effect of radiation destroys the tumor cells, likewise extends to damage the healthy tissues in the areas which are being treated. The exposure to ionizing radiation increases the production of the reactive oxygen species (ROS) and this can lead the irradiated cells into a state of oxidative stress, or imbalance between ROS production and the detoxification process by the biological systems [8].

Numerous reports focused on the health promoting and antioxidant effects of grapes. Interest in the health benefits of grapes has been increased due to their high phenolics and essential fatty acids contents. Most phenolics in grapes were detected in the seeds. The Grape seed oil (GSO) contains high amounts of phenolic compounds, including gallic acid, catechin, epicatechin and procyanidins (composed of flavan-3-

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ols units, (+)-catechin and (−)-epicatechin, linked together through C4–C6 and C4–C8 inter-flavonoid bonds), resveratrol, minor amounts of hydroxytyrosol and melatonin. Further, a high level of the vitamin E (60–120 mg/100 g), in addition to a high amount of essential-polyunsaturated fatty acids such as, linoleic acid (69–78%), palmitic acid (5–11%), oleic acid (15–20%), and stearic acid (3–6%), other constituents like sugars and considerable amount of macro and micro elements such as phosphorus, potassium, calcium, magnesium, iron, zinc, copper and manganese were found in GSO [9–15]. The potent antioxidant property and the biological activity are claimed to be the protective mechanism of GSO [16–18]. Therefore, this study was carried out to investigate the protective effect of GSO on acute liver injury induced by carbon tetrachloride in γ-irradiated rats.

2. Materials and Methods

2.1. Chemicals

The GSO (*Vitis vinifera*) was obtained from Sigma Chemical Co., Nasr City, Cairo, Egypt. Carbon tetrachloride (CCl_4) was obtained from Merck. All other chemicals and reagents used in this study were of analytical grade.

2.2. Irradiation of Animals

Whole-body gamma-irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using Canadian Gamma Cell-40 biological irradiator (^{137}Cesium), manufactured by the Atomic Energy of Canada Limited, Ontario, Canada. The radiation dose rate was 0.456 Gy/min at the time of exposure. The total radiation dose was 7 Gy as a single dose of the whole body. Animals were not anesthetized before irradiation.

2.3. Experimental Animals

2.3.1. Animals

Female *Wistar* rats (weighing 100–120 g) were obtained from the Nile Pharmaceutical Co., Cairo, Egypt. They were housed at the animal facility at the National Center for Radiation Research and Technology. Upon arrival, the animals were allowed to acclimatize for one week before starting the experiment. The animals were kept under standard laboratory conditions of light/dark cycle (12/12 h), a temperature of 25 ± 2 °C and humidity of 60 ± 5%. The rats were housed in cages with free access to food and drinking water ad libitum. They were provided with a nutritionally adequate standard laboratory (pellet) diet. The study was conducted in accordance with international guidelines for animal experiments and approved by the Ethical Committee of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

2.4. Experimental Design

The rats were divided into the following groups, (6 animals/group):

Control group (C): rats administered water orally by gastric intubation every day for seven days.

Irradiated group (R): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation [19], then, they were administered water orally every day for seven days.

GSO treated group (GSO): Rats were orally administered 3.7 g/kg body weight (4 ml/kg b. wt.) of GSO [18], till the end of the experimental period.

CCl_4 treated group (CCl_4): rats were orally administered water, then they were intraperitoneally (IP) injected with acute single dose; 2 ml/kg body weight of CCl_4 (prepared with olive oil 1:1 v/v) after 7 days from the beginning of the experiment [20].

Irradiated/CCl_4 treated group (R/CCl_4): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation. Irradiated rats were orally administered water by gastric intubation every day for seven days, then they were administered intra-peritoneally (IP) with an acute single dose; 2 ml/kg body weight of CCl_4 (prepared with olive oil 1:1 v/v).

Irradiated/GSO treated group (R/GSO): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation, 2 h later, rats were orally administered 3.7 g/kg body weight (4 ml/kg b. wt.) of GSO, every day till the end of the experimental period.

GSO/CCl_4 treated group (GSO/CCl_4): rats were orally administered 3.7 g/kg body weight (4 ml/kg b. wt.) of GSO, every day for seven days. Then they were administered IP with acute single dose, 2 ml/kg body weight of CCl_4 (prepared with olive oil 1:1 v/v).

Irradiated/GSO/CCl_4 treated group (R/GSO/CCl_4): rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation; 2 h later, rats were orally administered 3.7 g/kg body weight (4 ml/kg b. wt.) of GSO every day for seven days. Then they were administered IP with acute single dose, 2 ml/kg body weight of CCl_4 (prepared with olive oil 1:1 v/v).

After 16 h of CCl_4 administration [20]; including an overnight fasting, with free access to drinking water, blood samples were collected from the retro-orbital venous plexus of the eye of each animal using a glass capillary tube under light ether anesthesia. The blood was collected in glass-tubes free of anticoagulant, allowed to clot, centrifuged at 1200 g using universal centrifuge (16R, Germany) and sera were separated. The liver of each animal was excised immediately and washed with physiological saline and stored at −80 °C.

2.5. Determination of Liver Transaminases

ALT and AST were determined in serum, using a biochemical blood analyzer (Alfa Wassermann Diagnostic Technologies, LLC, ACE, Alera, USA), at the Regional Center for Food and Feed (RCFF), Agriculture Research Center.

2.6. Determination of the Oxidative Stress Parameters and the Antioxidant Enzymes in the Liver Homogenate

Part of the liver was weighed and homogenized (10%) in chilled 50 mmol phosphate buffered saline (pH 7.4), centrifuged at 1200 g, at 4 °C for 15 min, using universal centrifuge (16R, Germany), then the supernatants were used for the determination of the following parameters:

LPO, in terms of malondialdehyde (MDA) was measured according to the method of Satoh [21], using 1, 1, 3, 3-tetraethoxypropane as a standard. MDA concentration was expressed as nmol/g wet tissue.

Nitric oxide (NO) was determined as nitrite concentration. The method used depends on Griess reaction, which converts nitrite into a deep purple azo-compound which photometrically measured at 540 nm according to the method of Montgomery and Dymock [22]. NO concentration was expressed as μmol/g wet tissue.

GSH concentration was measured according to Beutler et al. [23] using DTNB and was expressed as mg/g wet tissue.

Superoxide dismutase (SOD) activity was determined according to Nishikimi and Yogi [24]. The assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye, which was followed photometrically at 560 nm. The enzyme activity was expressed as U/g wet tissue.

Catalase (CAT) activity was assessed according to Aebi [25]. Catalase reacts with a known quantity of hydrogen peroxide (H_2O_2) in the