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Protective effect of *Withania somnifera* against radiation-induced hepatotoxicity in rats

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ABSTRACT

The aim of this study was to investigate the protective effect of root extract of *Withania somnifera* (WS) against gamma-irradiation-induced oxidative stress and DNA damage in hepatic tissue after whole body gamma-irradiation. Fourty male albino rats were divided into four groups. In the control group, rats were administered vehicle by tube for 7 consecutive days. The second group were administered WS (100 mg/kg, by gavage) for 7 consecutive days. Animals in the third group were administered vehicle by tube for 7 consecutive days, then exposed to single dose gamma-irradiation (6 Gy). The fourth group received WS for 7 consecutive days, one hour later rats were exposed to gamma-irradiation. Irradiation hepatotoxicity was manifested biochemically by an increase in hepatic serum enzymes, significant elevation in levels of malondialdehyde (MDA) and total nitrate/nitrite NO(x), significant increase in heme oxygenase activity (HO-1), as well as a significant decrease in reduced glutathione (GSH) content and the activities of antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) in hepatic tissues. Marked DNA damage was observed. WS pretreatment showed significant decrease in serum hepatic enzymes, hepatic NO(x) and MDA levels and DNA damage, significant HO-1 induction and significant increase in SOD, GSHPx activities and GSH content compared to irradiated group. These observations suggest that WS could be developed as a potential preventive drug for ionizing irradiation induced hepatotoxicity disorders via enhancing the antioxidant activity and induction of HO-1.

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1. Introduction

At the present time, more than one-half of all cancer patients are treated with radiation therapy. Radiation causes tissue injury, both in tumors and in normal tissues, by induction of apoptosis or clonogenic cell death triggered by free radical-mediated DNA damage (Vozenin-Brotons, 2007). The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) as a result of water radiolysis. ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy (Fang et al., 2002). These ROS attack cellular macromolecules like DNA, RNA, proteins, membranes, causing their dysfunction and damage (Chittezhath and Kuttan, 2006).

Heat-shock proteins (stress proteins) are among important cell response proteins that are present in cells under normal conditions. They are expressed at high levels under stress conditions, such as heat–cold shock, inflammation and oxygen deprivation (Papp et al., 2003). Heme oxygenase (HO) is the first and the rate limiting enzyme in the catabolism of heme (Maines, 2005) to yield equimolar amounts of biliverdin, carbon monoxide and free iron. Three isoforms of HO designated as HO-1, HO-2 and HO-3 have been identified in mammals (Immenschuh and Ramadori, 2000). HO-1 is also known as heat shock protein 32 (Ryter et al., 2006). HO-1 is the inducible isoform that is thought to be a homeostatic and protective gene against various stress-related conditions (Otterbein et al., 2011), including oxidative stress, hemin and other none-heme agents such as ultraviolet (Ossola and Tomaro, 1998), carbon tetrachloride treatment (Hwang et al., 2009), glutathione depletion (Oguro et al., 1996) and endotoxemia (Dorman et al., 2004). It is worth noting that any of these stimulants have the capacity to generate ROS. As a defense against such toxicity, normal cells increase HO-1 synthesis to protect the organism against oxidant stress besides the regulation of antioxidant defense mechanism (Ryter et al., 2006). Induction of HO-1 is entirely prevented by administration of several antioxidants such as α-tocopherol and allopurinol (Tomaro et al., 1991).

Withania somnifera (L.) Dunal (ashwagandha, Indian ginseng, WS) is a perennial plant belonging to the order Solanaceae, is

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widely used in Ayurvedic medicine (Sangwan et al., 2007). The pharmacological effects of the roots of WS are attributed to the presence of withanolides, a group of steroidal lactones (Udayakumar et al., 2009). It promotes physical and mental health, rejuvenates the body in debilitated conditions, and increase longevity (Kulkarni and Dhir, 2008). WS is known to have anti-inflammatory (Alhindawi et al., 1992), antitumor (Widodo et al., 2010), antidiabetic (Prasad et al., 2010), antioxidant (Das et al., 2010), cardioprotective (Deocaris et al., 2008) and antistress effect (Udayakumar et al., 2010). It has the potential to increase tumor sensitization to radiation and chemotherapy while reducing some of the most common side effects of these conventional therapies (Sharada et al., 1996). Therefore, the present study was aimed at determining the hepatoprotective effects of WS extracts on ionizing radiation-induced oxidative stress.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats, weighing 120–150 g were obtained from the experimental animal house of the National Cancer Institute (NCI), Cairo University. Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water ad. Libitum. Animals were kept under a controlled lighting condition (light: dark, 13 h–11 h). The animals' treatment protocol was approved by the animal care committee of the National Cancer institute, Cairo, Egypt, following the guidelines of National Institutes of Health (NIH).

2.2. Chemicals

Ethylene diamine trichloroacetic acid (EDTA), nitroblue tetrazolium (NBT), Greiss reagent, trichloro-acetic acid (TCA), thiobarbituric acid (TBA), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) were procured from Sigma Chemical Co., St. Louis, USA. All other chemicals and solvents used were of the highest purity grade available. *Withania somnifera* (WS) extract manufactured by Idea Sphere Inc., American Fork, USA. The alcoholic extract was filtered through a Whatman filter paper #4 and evaporated in a rotary evaporator under reduced pressure at 60 °C, which was stored in refrigerator for further use (Senthilnathan et al., 2006). The required amount was suspended in 0.5 ml of double distilled water (DDW) (Balgia et al., 2004).

2.3. Irradiation

Whole-body gamma-irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, using an AECL (137 cesium) Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.012 Gy/s.

2.4. Experimental design

Male albino rats were divided into four groups, 10 rats in each. In the control group, rats were administered vehicle (0.5 ml of DDW) by gavage for 7 consecutive days. The second group were administered WS (100 mg/kg, by gavage) for 7 consecutive days (Rajasankar et al., 2009). Animals in the third group were administered vehicle by gavage for 7 consecutive days, then exposed to single dose γ-irradiation (6 Gy). The Fourth group were received WS (100 mg/kg, by gavage) for 7 consecutive days, one hour later rats were exposed to single dose γ-irradiation (6 Gy) (Mansour, 2006).

2.5. Biochemical assays

Twenty-four hrs after the last specific treatment, animals were anesthetized with ether, and blood samples were obtained by heart puncture. Serum samples were separated for the measurement of indices of hepatotoxicity. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were estimated according to the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were estimated according to the methods of Roy et al. (1970) and Young (1990), respectively. Animals were then sacrificed by decapitation after exposure to ether in a dessicator kept in a well-functioning hood. Livers were removed and washed with ice-cold saline, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific, Danbury, Conn., USA). The homogenates were centrifuged at 800 g for 5 min at 4 °C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 min at 4 °C to get the post mitochondrial supernatant which was used to assay superoxide dismutase (SOD) activity.

In the liver homogenate, reduced glutathione (GSH) was determined spectrophotmetrically according to the methods of Ellman (1959) using Ellman's reagent [5,5′-dithio-bs- (2nitrobenzoic acid)] The results were expressed as μmol/g tissue. Malondialdehyde (MDA) levels in liver tissue homogenates were determined spectrophotometrically using the method of Buege and Aust (1978). The results were expressed as nmol/g tissue. Glutathione peroxidase (GSH-Px) was determined in liver homogenate according to the method of Lawrence and Burk (1976). The changes in the absorbance at 340 nm were recorded at 1-minute interval for 5 min and an extinction coefficient of $6.22 \times 10^{-}3$ was used for calculation. The results were expressed as umol/min/gm tissue. Superoxide dismutase (SOD) activity in liver homogenate was determined according to the method of Minami and Yoshikawa (1979). This method is based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium. The formazan color developed was determined spectrophotometrically (Spectronic 501, Shimadzu, Japan). Enzymatic activity was expressed as μg/g of tissue. Total nitrate/nitrite (NO(x)) level was determined according to the method of Ignarro et al. (1987). The assay is based on the diazotization of sulfanilic acid with nitric oxide at acidic pH and subsequent coupling with N-(10-naphthyl)-ethylenediamine to yield an intensely pink colored product that is measured spectrophotometrically at 540 nm. The levels of NO(x) were expressed as nmol/g tissue. Tissue HO-1was extracted according to the method described by Takeda et al. (2000) and Morsi et al. (2006). 1 mL of the homogenate was mixed with 2.5 mL of Tris–HCl buffer, pH 7.6 (10 mM containing 250 mM sucrose and 0.4 mM phenyl methyl sulfonyl fluoride). To separate the mitochondrial pellet, the homogenate was centrifuged at 800*g* for 10 min and then at 13,500*g* for 20 min at 4°C. The resulting supernatant was used for the assay of HO-1 protein level. HO-1expression was quantified by means of a commercially available rat HO-1 enzyme-linked immunosorbent assay (ELISA) kit, according to the method of Katori et al. (1999). HO-1 protein level was determined by means of a standard curve constructed using serial dilutions of recombinant rat HO-1 protein.

2.6. Analysis of DNA fragmentation: agarose gel electrophoresis

According to the method of Katoh et al. (1996), the liver tissue was homogenized and lysed in a cold lysis buffer (10 mM Tris–HCl, 5 mM disodium EDTA, and 0.5% Triton X-100, pH 8.0) for 10 min at 4 °C. The DNA was sequentially extracted twice using half volumes of phenol/chloroform and incubated at 55 °C for 10 min. After centrifugation at 3000 rpm for 20 min, the upper layer was incubated with proteinase K at 37 °C for 60 min followed by incubation with ribonuclease at 37 °C for 60 min. The DNA was precipitated by adding 0.1 vol of 10 M ammonium acetate and 2.5 vol of 100% ethanol and maintained at 20 °C overnight. DNA was collected by centrifugation at 15,000g for 20 min, air-dried, and resuspended in TE buffer (10 mM Tris–HCl, 5 mM EDTA, pH 7.4). Agarose gel electrophoresis was carried out for the analysis of DNA fragmentation (Yokozawa and Dong, 2001). The resulting DNA preparations were electrophoresed through a 1.4% agarose gel containing ethidium bromide using TBE buffer (Tris–boric acid–EDTA buffer, pH 8.3) at 40 V for 5 h. Equal quantities of DNA (based on optical density measurements at 260 nm) were loaded in each lane, and a molecular DNA marker was used as a molecular mass standard. DNA fragmentation was visualized and photographed under ultraviolet illumination.

2.7. Statistical analysis

Differences between obtained values (mean ± SE, $n=10$) were carried out by one way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. A p-value of 0.05 or less was taken as a criterion for a statistically significant difference.

3. Results

Gamma-irradiation (6 Gy) induced a significant increase in the activity levels of serum AST (51.7%) and ALT (131.75%) compared to control group ($P < 0.001$). Treatment with WS ameliorated the effect of radiation exposure. Gamma-irradiation (6 Gy) induced a significant increase in ALP (44.3%) and GGT (313.3%) activities compared to control group. Administration of WS prior to irradiation induced a significant decrease in ALP (10.1%) and GGT (99.73%) activities compared to irradiated group (Table 1).

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