Biomedicine & Aging Pathology 4 (2014) 265–272

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Original article

Modulatory effect of *moringa oleifera* against gamma-radiation-induced oxidative stress in rats

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ARTICLE INFO

Article history:
Received 24 March 2014
Accepted 18 April 2014
Available online 24 May 2014

Keywords:
Ionizing radiation
Moringa oleifera
Lung
Heart
Oxidative stress

ABSTRACT

The present study was carried out to investigate the antioxidant activity and the protective effects of the aqueous leaf extract of *Moringa oleifera* (MO) on α-radiation-induced toxicity in cardiac and pulmonary tissues in rats. Rats were administered MO (300 mg/kg, oral gavage) for 15 consecutive days and 1 h after the last dose, rats were exposed to 6 Gy α-radiation. Irradiation toxicity was manifested biochemically by an increase in serum triglycerides, total cholesterol and low density lipoprotein-cholesterol, creatine phosphokinase and lactate dehydrogenase activities, elevation of MDA and NO(x) in examined tissues as well as a decrease in serum level of high density lipoprotein-cholesterol and tissue glutathione(GSH) level, catalase(CAT), glutathione peroxidase(GSHPx) and superoxide dismutase(SOD) activities. Marked DNA damage was observed. Pretreatment with MO showed a significant amelioration in the levels of lipid profiles, MDA, NO(x) and DNA damage. The antioxidant enzymes increased significantly along with the levels of GSH. The aqueous extract of MO exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical. Moreover, histopathological examination of heart and lung tissues confirmed the biochemical data. Our results show that pretreatment with MO extract minimize the oxidative stress of α-irradiation indicating its free radical scavenging and potent antioxidant activities.

1. Introduction

Radiotherapy has evolved to become one of the bases of treatment for various types of cancers and it is estimated that approximately 50% of cancer patients derive benefits from it [1]. However, radiation-induced normal tissue damage restricts the therapeutic doses of radiation that can be delivered to tumors and thereby limits the effectiveness of the radiotherapy. Tissue injury from ionizing radiation ultimately begins with oxidative stress from radiolysis of water and the formation of reactive oxygen species (ROS) [2]. These ROS are highly reactive molecules and can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins and membranes [3]. However, ionizing radiation decreases the total antioxidant capacity of the organism and results in an imbalance between pro-oxidants and antioxidants [4]. The imbalance between the exposure to oxidants and the endogenous antioxidants results in oxidative stress, which has been correlated with many pathological conditions, including cancer, pulmonary, cardiac, immunosuppression and neurodegenerative diseases [5,6].

Radiation-induced lung injury and cardiac complications is a disabling and potentially fatal, dose-limiting toxicity of thoracic radiotherapy for lung cancer, breast cancer, lymphoma, esophageal cancer [7–11], and total body radiation [12]. Thus, drugs that scavenge or inhibit the formation of ROS may have relevance to cancer patients by ameliorating damage of normal tissues exposed to radiotherapy [13].

Moringa oleifera (MO) is an edible plant. *M. oleifera*, Lam (*M. oleifera*), is a member of the *Moringaceae* family of perennial angiosperm plants, distributed in many countries of the tropics and subtropics. A wide variety of nutritional and medicinal benefits have been attributed to its roots, bark, leaves, flowers, fruits, and seeds [14–19]. Phytochemical studies have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids [20–22].

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http://dx.doi.org/10.1016/j.biomag.2014.04.006
2210-5220/

Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the native system of medicine [14,23].

This study has been initiated to investigate the antioxidant activity and the possible protective effects of MO against γ-irradiation-induced oxidative damage in lung and heart tissues in rats.

2. Materials and methods

2.1. Animals

Adult male albino 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 the National Institutes of Health (NIH).

2.2. Chemicals

The aqueous leaf extract of *M. oleifera* (MO) was purchased from Genesis Today, Inc., USA. The contents of the capsule were reconstituted in saline solution immediately before orally administered to the animals in order to attain the required dose. Greiss reagent, trichloro-acetic acid (TCA), nitroblue tetrazolium (NBT), 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.

2.3. Irradiation

Whole body α-irradiation was performed at the National Centre 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. Determination of antioxidant activity by the DPPH radical scavenging method

Measurement of free radical scavenging activity of MO on DPPH radical was determined according to the method described by [24]. Briefly, 1.5 mL of DPPH solution (0.1 mM, in 95% methanol) was incubated with varying concentrations of MO. The reaction mixture was shaken well and incubated for 15 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank (control). Ascorbic acid was used for comparison as antioxidant materials. The radical scavenging effect was measured as a decrease in the absorbance of DPPH and can be calculated using the following equation:

Scavenging effect (%) = [1 − (A samples/A control)] × 100.

From this experiment, the 50% DPPH radical scavenging concentrations (IC_{50}) of the test substances were calculated.

2.5. Experimental design

Male albino rats were divided into four groups, 6 rats in each. In the control group, rats were administered saline solution by tube for 15 consecutive days. The second group was administered MO extract (300 mg/kg, by gavage) for 15 consecutive days [25]. Animals in the third group were administered saline by tube for 15 consecutive days, then exposed to single dose γ-irradiation (6 Gy) The Fourth group was received a MO extract (300 mg/kg, by gavage) for 15 consecutive days, one hour later rats were exposed to single dose γ-irradiation (6 Gy). Twenty-four hours after the last dose of the specific treatment, animals were anesthetized with ether and were then sacrificed by decapitation. Twenty-four hrs after the last specific treatment, the rats were sacrificed. Blood samples were immediately collected into centrifuged tubes, kept at room temperature for one hour and centrifuged at 3 000 RPM for 20 min, to obtain serum for biochemical assays, the serum was kept at −20 until used. Lungs and hearts were quickly excised, washed with saline, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA) to make 20% homogenate.

2.6. Biochemical parameters

2.6.1. Serum lipid profiles, creatine phosphokinase and lactate dehydrogenase

The appropriate kits (Bio-Diagnostic, Dokki, Giza, Egypt) were used for the measurements of serum triacylglycerides, low density lipoprotein-cholesterol (LDL), high density lipoprotein-cholesterol (HDL) and total cholesterol according to the methods described by Fossati and Prencipe [26], Wieland and Seidel [27], Lopez-Virella et al. [28] and Allain et al. [29], respectively. Serum creatine phosphokinase (CPK) and Lactate Dehydrogenase were determined according to the methods of Swanson and Wilkinson [30] and IFCC [31], respectively.

2.6.2. Estimation of malondialdehyde and total nitrate/nitrite (NO(x)) levels

In the lung and heart homogenates, malondialdehyde (MDA) and total nitrate/nitrite (NO(x)) levels, an index of lipid peroxidation, in tissue homogenates were determined spectrophotometrically using the method of Buege and Aust [32] and Ignarro et al. [33], respectivey. The results were expressed as nmol/g tissue.

2.6.3. Determination of reduced glutathione content

Reduced glutathione (GSH) was determined spectrophotmetrically according to the methods of Ellman [34] using Ellman's reagent [5,5'-dithio-bis-(2-nitrobenzoic acid)]. The results were expressed as μmol/g tissue.

2.6.4. Estimation of superoxide dismutase, glutathione peroxidase and catalase activities

Superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT) activities in tissue homogenate were determined according to the method of Minami and Yoshikawa [35], Lawrence and Burk [36] and Aebi [37], respectively.

2.7. Analysis of DNA fragmentation: agarose gel electrophoresis

According to the method of Katoh et al. [38]. the lung and heart tissue were 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