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Cinnamon extract ameliorates ionizing radiation-induced cellular injury in rats

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ABSTRACT

The present study aimed to investigate the protective role of cinnamon extract against inflammatory and oxidative injuries in gamma irradiated rats. Rats were subjected to fractionated doses of gamma radiation. Cinnamon extract were daily administrated before starting irradiation and continued after radiation exposure. The results obtained revealed that the administration of cinnamon extract to irradiated rats significantly ameliorated the changes induced in liver antioxidant system; catalase, superoxide dismutase and glutathione peroxidase activities as well as reduced glutathione concentration. The liver's lipid peroxidation and protein oxidation indices were significantly decreased when compared with their equivalent values in irradiated rats. Furthermore, the changes induces in xanthine oxidoreductase system were significantly diminished. In addition, the changes in liver nitric oxide contents, serum tumor necrosis factor alpha and C-reactive protein levels were markedly improved. In conclusion, the administration of cinnamon extract might provide substantial protection against radiation-induced oxidative and inflammatory damages.

1. Introduction

Ionizing radiation (IR) has attracted a lot of attention due to its beneficial as well as possible harmful effects to human population (Jagetia et al., 2003). Ionizing radiation is known to induce oxidative stress through generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant in the cells, which is suggested to culminate in cell death (Srinivasan et al., 2007).

The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by body's defense systems. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues (Shrinivas et al., 2000). Radiation exposure attenuates endogenous antioxidant enzymes, which are considered to function as part of a first line defense mechanism to maintain redox balance and normal biochemical processes. Thus, supplementation of antioxidants to improve the efficacy of radiotherapy is a current proposed strategy, as antioxidants are capable to scavenge free radicals from the radiolysis of water and to protect cells from damage (Barker et al., 2005).

Studies on plant extracts and phytochemicals as modifiers of radiation effects are a new area of research (Shimoi et al., 1996; Goel et al., 2004). Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites, which are rich in antioxidant activity (Cai et al., 2004).

Cinnamon is one of the most widely used herbal medicines with diverse bioactive effects, belonging to the Lauraceae family (Jayaprakasha et al., 2003). *Cinnamomum cassia* is also named Chinese cinnamon, is the outer skin of an evergreen tall tree, which contains large amounts of bioactive molecules including essential oils, tannin, mucus and carbohydrates (Kwon et al., 2009).

The main active ingredient of cinnamon is considered to be doubly linked A type polyphenols, which are A type doubly linked procyanidin oligomers of the catechins and/or epicatechins. These polyphenolic polymers found in cinnamon may function as antioxidants, potentiate insulin action, and may be beneficial in the control of glucose intolerance and diabetes (Anderson et al., 2004). Also, cinnamaldehyde, one of major cinnamon contents, found to reduce LPS-induced NF-κB transcriptional activity through the inhibition of DNA binding activity in macrophages (Reddy et al., 2004). In addition, cinnamaldehyde found to reduce IL-1β-induced cyclooxygenase-2 activity in endothelial cells and

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to exert several biological effects such as anti-angiogenic activity and immunomodulating activity (Guo et al., 2006).

This study has been oriented to investigate possible protective mechanisms of cinnamon extract against gamma irradiation-induced oxidative damage and inflammatory cascades.

2. Materials and methods

2.1. Preparation of water extract of cinnamon

Dried *C. cassia* bark available in local market was grounded to a fine powder. Water extracts of the cinnamon was freshly prepared by soaking 10 gm of the ground spice in 100 ml distilled water at 90 °C for 2 h (Kreydiyyeh et al., 2000). The aqueous extract was filtered and the supernatant collected. The dry yield was obtained by drying the filtered extract by dehydration in oven at 80 °C overnight. The resulting dark reddish brown dry extract was weighed and the dry yield calculated.

2.2. Animals

Male Wistar rats (weighing 100–120 g) were obtained from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Upon arrival, the animals were allowed to acclimatize for 1 week before starting the experiment. Food and water were available throughout the experiment *ad libitum*. Animal experimentation were consistent with the guidelines of Ethics by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) in accordance with the recommendations for the proper care and use of laboratory animals approved by animal care committee of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

2.3. Irradiation

Whole-body gamma-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (^{137}cesium) Gamma Cell-40 biological irradiator. The dose rate of the gamma source was 0.46 Gy min^{-1}. Animals were not anesthetized before irradiation.

2.4. Experimental design

The experiment duration divided into two stages, (*pre-irradiation stage*) 14 days before irradiation and the other 14 days (*radiation exposure stage*) started post exposure to the first dose of γ-irradiation until the end of the experiment. Rats that were divided into four groups ($n=6$). Group I (*Control group*), included rats neither treated nor irradiated. Rats of this group are received orally an equivalent volume of distilled water (vehicle of cinnamon) during the period of cinnamon administration. Rats in group II (*Irradiated group*) were exposed to 4 fractions of gamma radiation (2 Gy dose every 4 days) and like control group rats of this group received orally an equivalent volume of distilled water during the period of cinnamon administration. In group III (*Cinnamon group*), rats were administered 200 mg/kg/day cinnamon aqueous extract (Kim et al., 2006) via oral tube along the experimental period. Group IV (*Cinnamon Irradiated*) included rats that were administered 200 mg/kg/day cinnamon aqueous extract orally for 14 days before gamma irradiation, and the administration of the extract was extended during radiation exposure period (14 days; 2 Gy every four days up to the total dose of 8 Gy). Rats were sacrificed on the 3rd day post last radiation fraction and subjected to liver and serum biochemical measurements.

2.5. Biochemical assays

After an overnight fast, rats were anesthetized with ether and then sacrificed. Blood samples from each rat were collected by retro-orbital puncture using blood capillary tubes. Serum was obtained immediately by centrifugation of blood samples at 3000 rpm for 10 min. Liver was directly separated after sacrificing, washed in ice-cold saline then the liver samples were homogenized in doubly distilled water (10% W/V) using homogenizer then the cell debris was removed by centrifugation at 3000 rpm for 10 min. The homogenates supernatant were subjected to the following biochemical analysis.

Xanthine oxidoreductase system (XOR), including XD and XO activities (U/mg protein) were assayed by measurement of uric acid formation in the presence or absence of NAD^+ at 37 °C, as described by Waud and Rajagopalan (1976). XO and XD activities were expressed as nmole uric acid formed per mg protein. Superoxide dismutase (SOD) activity was assayed via the method of Kakkar et al. (1984), based on the inhibition of superoxide ions generated by phenazine methosulfate that converts nitroblue tetrazolium (NBT) to NBT-diformazan, which absorbs light at 560 nm. SOD activity was defined as the amount of enzyme required to give 50% inhibition of NBT reduction and expressed as units/min/mg protein. Catalase (CAT) activity was assayed using the method of Sinha, (1972), based on the utilization of H_2O_2 by the enzyme. One unit of the enzyme is expressed as μmoles of H_2O_2 utilized per minute per mg protein. The GSH content was determined photometrically at 412 nm using 5, 5′-dithiobis-2-nitrobenzoic acid (Ellman, 1959). Glutathione peroxidase (GPx) activity (U/mg protein) was assayed according to the method of Gross et al. (1967). The activity of GPx expressed as GSH consumed per min per mg protein. The protein content was determined according to the Folin–Lowry method (Lowry et al., 1951). Lipid peroxidation product, malondialdehyde (MDA), was measured (nmole/g wet tissue) by thiobarbituric acid assay, which is based on MDA reaction with thiobarbituric acid forming thiobarbituric acid reactive substances (TBARS), a pink colored complex exhibiting a maximum absorption at 532 nm (Yoshioka et al., 1979). The protein carbonyl content (μmole/mg protein) was estimated by the method of Julie et al., (1999). The photometric carbonyl assay is based on the reaction of 2, 4-dinitrophenylhydrazine with carbonyl groups to produce 2, 4-dinitrophenyl-hydrazone. The levels of serum TNF-α (pg/ml) were measured by enzyme-linked immunosorbent assay (ELISA) kit (BMS 622, Vienna, Austria), following the manufacturer's instructions. Also, serum CRP levels (mg/l) were measured by enzyme-linked immunosorbent assay (ELISA) kit (BioCheck, USA), following the manufacturer's instructions. Nitric oxide (NO) was measured (nmole/g wet tissue) as stable end product, nitrite, according to the method of MarMiranda et al. (2001). The assay is based on the reduction of nitrate by vanadium trichloride combined with detection by the acidic Griess reaction. The diazotization of sulfanilic acid with nitrite at acidic pH and subsequent coupling with *N*-(10 naphthyl)-ethylenediamine produced an intensely colored product that is measured at 540 nm.

2.6. Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) and post hoc Duncan test using the Statistical Package for the Social Sciences (SPSS) Version 15.0 for windows. The values are mean ± S.E. for six samples in each group. Values were considered statistically significant at $P<0.05$.

3. Results

Fractionated 8 Gy gamma-irradiation induced a significant increase (124.6%) in xanthine oxidase (XO) activity paralleled with significant decrease (−44.1%) in xanthine dehydrogenase (XD) activity compared to control group. Treatment with cinnamon extract (200 mg/kg) ameliorated the effect of radiation exposure as shown by dropped XO and increased XD activities (percentage changes from control reached 63% and −29.4%, respectively), although XO activity was still significantly higher than the control values (Table 1).

The effects of radiation on endogenous antioxidant status are shown in Table 2. Gamma-irradiation induced significant decrease in the hepatic CAT, GPx activities and GSH content and significant increase in SOD activity in liver compared with control

Table 1
Xanthine oxidase (XO) and xanthine dehydrogenase (XD) activities in the different animal groups.

	Control	Cinnamon	Irradiated	Cinnamon-Irradiated
XO (U/mg protein)	2.11 ± 0.09	1.95 ± 0.09 [b] (−7.6%)	4.74 ± 0.32[a] (124.6%)	3.44 ± 0.41[a, b] (63%)
XD (U/mg protein)	5.00 ± 0.18	5.45 ± 0.16[b] (6.8%)	2.85 ± 0.16[a] (−44.1%)	3.60 ± 0.32[a, b] (−29.4%)
XO/XD ratio (%)	42.2%	35.7%	166.3%	95.5%

All values are expressed as mean ± SE of 6 animals, % change from control.

[a] Significant ($P<0.05$) when compared with the control group.
[b] Significant ($P<0.05$) when compared with the irradiated group.