

Radioprotective Effects of Dietary Ginger (*Zingiber officinales* Rosc.) Against Fast Neutron-induced Oxidative Stress in Rats

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Abstract: The radioprotective effect of *Zingiber officinales* (ginger) against fast neutron-induced oxidative stress was investigated in male albino rats. Various antioxidant parameters such as glutathione peroxidase (GPx), glutathione reductase (GR) enzyme activities and lipid peroxidation (LPO) were assayed before and after whole body exposure to fast neutrons of fluence 10^5 n/cm². Moreover, the same parameters were investigated in irradiated rats pretreated with ginger. Irradiation clearly decreased GPx and GR activity but ginger treatment before irradiation obviously countered such decrease in the activity of these enzymes. Radiation-induced high LPO which reduced by ginger treatment before irradiation. Consumption of ginger extract resulted in reductions of plasma total cholesterol in ginger group (by 17%) and in irradiated group (8%). This study implies that dietary ginger offers radioprotection at biochemical level by protecting antioxidant enzymes, reducing LPO and inhibiting the synthesis of cholesterol.

Key words: Ginger • Oxidative stress • Antioxidant enzymes • Cholesterol • Fast neutrons

INTRODUCTION

Ginger, which is the underground stem or rhizome of the plant *Zingiber officinale* Roscoe, has been used as an important cooking spice around the world over 2000 years [1]. Its roots and the obtained extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity [2,3].

There are more than 50 antioxidants isolated from rhizomes of ginger [4]. The isolated antioxidants are divided into two groups; gingerol related compounds and diarylheptanoids. The nonvolatile fraction of the dichloromethane extract of ginger rhizomes exhibited a strong antioxidant activity. The fraction was purified by chromatographic techniques to provide five gingerol related compounds and eight diarylheptanoids [5]. Among them, 12 compounds exhibited higher antioxidant activity than α -tocopherol. The activity was probably dependent upon side chain structure and substitution patterns on the benzene ring. The oleoresin, responsible to the pungent flavor of ginger, varies from 4.0-7.5% and also possesses substantial antioxidant activity [6].

Antioxidants have been studied for their capacity to reduce the cytotoxic effects of radiation in

normal tissues for at least 50 years. Early research identified sulfur-containing antioxidants as those with the most beneficial therapeutic ratio, even though these compounds have substantial toxicity when given *in vivo*. Other antioxidant molecules (small molecules and enzymatic) have been studied for their capacity to prevent radiation toxicity both with regard to reduction of radiation-related cytotoxicity and for reduction of indirect radiation effects including long-term oxidative damage [7].

Pretreatment of mice with ginger reduced the severity of symptoms of gamma- radiation sickness and mortality at all the exposure doses and also increased the number of survivors in a ginger + irradiation group. Moreover, ginger treatment protected mice against gastrointestinal as well as bone-marrow-related deaths. Ginger was found to scavenge OH and O⁻² radicals in a dose-dependent manner *in vitro* [8, 9].

The radioprotective effects of ginger against exposure to fast neutrons have not been studied. Therefore, the aim of the present work was to investigate the radioprotective effects of ginger against fast neutron-induced oxidative stress in rats.

MATERIALS AND METHODS

Chemicals: Commercial ginger was purchased from Haraz company, Cairo, Egypt and all commercial kits used in this study were purchased from Biodiagnostics company, Cairo, Egypt.

Animals: Animals care and handling was done according to the guidelines set of the World Health Organization, Geneva, Switzerland [10]. 120 male Swiss albino rats weighing 150-180 g obtained from the Animal House of National Research Center were used in the present experiment. Rats were housed 8 per cage (50X40cm) at a constant temperature ($24\pm 2^\circ\text{C}$) with alternating 12 hours light and dark cycles and fed standard laboratory food and water *ad libitum*.

Treatment: Rats were fed orally 2% ginger solution once daily for 5 consecutive days before exposure to neutron-radiation.

Experimental Design: To achieve the ultimate goal of this study, 120 adult male rats were randomly divided into four groups of 30 animals each. The first group (C) served as a control and were fed normal diet with concurrent double-distilled water. The second group (G) were fed 2% ginger solution once daily for 5 consecutive days before blood collection. The third group (R) were fed normal diet with concurrent double-distilled water before exposure to neutron-radiation of fluence 10^5 n/cm^2 . The fourth group (G+R) were fed orally 2% ginger solution once daily for 5 consecutive days before exposure to neutron-radiation.

Exposure System: The personnel protective precaution was applied according to Spurny *et al.* [11]. The exposure system was at the Department of Biophysics, Cairo University. Fission neutrons from californium-252 (CF^{252}) source were used for the whole body irradiation of the experimental animals. The source was 50 μgm CF^{252} manufactured by the Radiochemical Center, Amersham in England. The half life of the source is 2.37 years. The original yield of the source was $1.4\times 10^6 \text{ n/sec}$ and the present yield is $5\times 10^6 \text{ n/sec}$. The average energy of the incident fission neutrons from the source is 2 Mev.

During the whole body irradiation, the animals were kept in a double walled thin wooden cage in separated chambers that permit them to move freely and the source was at center of the cage. The cover of the cage is also perforated. The average distance of the animal from the source was 23 cm. All exposures were achieved at the

same distance from the source to keep all irradiations at the same dose rate.

The neutron fluences at the positions of exposures of the animals were measured through the use of calibrated Li^6F (TL) and LR 115 solid state track (SST) detectors. The animals were exposed to the fluence 10^5 n/cm^2 shown above at a constant dose rate. This was achieved through changing only the exposure time of the animals.

Blood Collection: Animals were anesthetized and blood samples were collected by heart puncture at the end of experimental period. Heparin was used as anticoagulant. Part of blood was used for determination of lipid peroxidation (LPO) and the activity of antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase (GR). The other part was used for determination of plasma level of total cholesterol as well as, Na^+ and K^+ concentrations.

Analytical Procedures: Lipid peroxidation in plasma was measured as the amount of malondialdehyde (MDA) formed employing thiobarbituric acid as described by Dursun, [12] using spectrophotometer (UV-1601 Pc, UV-Visible spectrophotometer, Shimadzu). GPx and GR activities were quantified spectrophotometrically according to Pleban *et al.* [13] and Sadeghi *et al.* [14], respectively. The serum concentration of total cholesterol was determined according to the method of Charles *et al.* [15]. While the plasma concentrations of Na^+ and K^+ were determined spectrophotometrically according to the method of Pei *et al.* [16].

Statistical Analysis: Data are presented as means \pm S.E. Analysis of data was performed using one-way analysis of variance (ANOVA) according to statistical method by Snedecor and Cochran [17]

RESULTS

Activities of GPx and GR and concentration of MDA in plasma of experimental rats are shown in Table 1. A markedly decrease in activities of the two antioxidant enzymes and an increase in MDA concentration are observed in irradiated group without ginger treatment, the values of antioxidant enzymes activities for irradiated group pretreated with ginger (G+R group) are higher than that of irradiated group without ginger treatment (R-group) and are different from control group (C), while MDA concentration of G+R group is clearly lower than that of the R group and higher than that of the C group.

Table 1: Activities of glutathione reductase, glutathione peroxidase and concentration of malondialdehyde in plasma of control, G, R and G+R groups of rats

Group	GR (u/ml)	G-Px (u/ml)	MDA (mmol/dl)
C	51.8±2.31	73.5±3.42	15.88±0.66
G	52.7±2.54	75.6±3.51a	16.23±0.65a
R	49.1±1.69a	68.3±2.44a	23.41±0.78a
G+R	51.3±1.88b	74.2±2.87ab	16.75±0.13ab

C: control, G: a group of rats treated with ginger; R: a group of rats exposed to ionizing radiation; G+R: a group of rats pretreated with ginger and then exposed to ionizing radiation. a. significantly different from C group, b. significantly different from R group

Table 2: Plasma concentrations of total cholesterol, Na⁺ and K⁺ in control, G, R and G+R groups of rats

Group	Total cholesterol (mg/dl)	Na ⁺ (mmol/dl)	K ⁺ (mmol/dl)
C	62.22±0.88	93.85±1.67	7.98±0.23
G	51.43±2.21ab	94.31±0.62b	7.52±0.09a
R	68.78±1.31a	91.89±1.08ab	8.11±0.22a
G+R	63.13±0.59ab	95.66±0.09ab	8.64±0.31ab

Legends as in Table 1

As seen in Table 2, whole body exposure of rats to fast neutrons of fluence 10^5 n/cm² led to an increase in the plasma concentration of total cholesterol. Pretreatment with ginger before irradiation led to a reduction of total cholesterol concentration.

The values of total cholesterol concentration of the G+R group is markedly higher than that of the C group. The values of plasma concentration of cholesterol of the control group pretreated with ginger before blood collection (G-group) is lower than that of the control group without ginger treatment. The values of Na⁺ and K⁺ concentrations for the G+R group is higher than those of the C and R groups, respectively.

DISCUSSION

Fast neutrons carry no electric charge and therefore their interaction with matter results from direct collision processes with atomic nuclei. Fast neutron (> 0.02 Mev) interacts mainly by elastic collision with the nuclei resulting in the so called nuclear recoils. Maximum energy will be transferred from fast neutrons to recoil nuclei for a head on collision with hydrogen. In living tissue with its high density of hydrogen atoms these interactions are of great importance. Recoil protons of energies up to that of the incident neutron are produced and being heavy charged particles, they cause intense ionization as they

slowed down. Neutrons also collide with other atomic nuclei (e.g. carbon, oxygen, etc.), producing highly energetic heavily ionizing nuclear recoils resulting in considerable biological damage. Recoil protons and highly energetic heavily ionizing nuclear recoils interact with the living matter by ionizing water molecules, producing secondary electrons. In indirect action of neutron radiation, the secondary electron interacts with a water molecule to produce a hydroxyl radical (OH \cdot), which in turn produces the damage to the biological macromolecules [18]. These free radicals inactivate GPx and GR enzymes and increase lipid peroxidation, as observed in the present study. The results of the oxidative stress and antioxidant enzymes in this work revealed that there was an increase in oxidative stress, malondialdehyde which is lipid peroxidation marker and a markedly decrease in the activities of antioxidant enzymes in albino rats irradiated with fast neutrons.

Antioxidant enzymes perform multifunctional activities to attenuate the radiotoxicity e.g. maintaining thiol-disulphid balance. In cells glutathione is maintained in the reduced form by the enzyme glutathione reductase (GR) and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants [19]. The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [20].

Ginger elevated GPx and GR enzyme activities and therefore reduces H₂O₂ concentration and consequently OH produced by Fenton reaction and thereby reducing the level of lipid peroxidation, as observed in the present study.

Proteins and enzymes are the core functional units in living systems and have tremendous roles in biological functions because most of the biological reactions are catalyzed by enzymes. When ionizing radiation, e.g. gamma rays or neutrons, impinge on biological systems, cells are damaged to different extents depending on radiation sensitivity. These ionizing radiations denature proteins and causes a conformational change in the structure which render them inactive. Examples of these proteins are GPx and GR enzymes which become inactive when exposed to ionizing radiation, as observed in the present study. Another example of these proteins is cholesterol-7 α -hydroxylase, the rate-limiting enzyme in bile acids biosynthesis, stimulating the conversion of cholesterol to bile acids. This liver enzyme become inactive when exposed to neutron radiation, leading to accumulation of cholesterol in the body, increasing their level in the plasma and impairing liver function, as

observed in the present study and in a previous study [21].

Pretreatment with ginger reduces the oxidative stress in the animals, by its high scavenging capacity of the ROS, protecting the antioxidant GPx and GR enzymes from being denatured and reducing further the oxidative stress marker (LPO), as observed in the present study. Reduced LPO has been observed in ginger treated rats compare to radiation control, which strengthen its radioprotective activity.

Pretreatment with ginger protects also the liver enzyme cholesterol-7 α -hydroxylase from being denatured, increasing its activity, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body. The hypocholesterolaemic effects of ginger have been observed in irradiated as well as unirradiated rats. Consumption of ginger extract resulted in reductions in plasma total cholesterol of control by (17%) and by (8%) in irradiated group. These hypocholesterolaemic effects of ginger have been observed also in previous studies [22-24].

High amounts of Na⁺ and K⁺ are found in ginger rhizomes [25]. This may account for the increased amounts of these ions in irradiated animals pretreated with ginger

CONCLUSION

The findings in this article suggest that a diet containing ginger is effective in exerting radioprotective effects by modulating oxidative stress and lowering the cholesterol biosynthesis.

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