The therapeutic effects of *Zingiber officinale* extract on mice irradiated by $^{60}$Co $\gamma$-ray

Yanyan Geng#, Xiaogang Du#, Xiaohan Cao, Yanger Chen, Huaiyu Zhang, Hanmei Liu, Zhiyu Chen and Xianyin Zeng*

Applied Biophysics and Immune Engineering Laboratory, College of Life Science, Sichuan Agricultural University, Ya’an 625014, Sichuan, China.

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Radiation can induce the acute and chronic radiation sickness via physical, chemical and biological mechanisms. In this study, we further investigated the therapeutic effects of gingerol on irradiated mice. Mice were given gingerol at 800 mg/kg B.W. by oral gavage once daily for 5 consecutive days after exposure to 1, 3, 5 and 7 Gy of $^{60}$Co-gamma-radiation. Gingerol treated mice after irradiated by 3 and 5 Gy significantly increased the spleen index compared to 3 and 5 Gy irradiation+DDW mice, respectively. Besides, the gingerol treatment facilitated the recovery of white blood cells (WBC), lymphocytes (LYM), red blood cell (RBC) and hemoglobin (HGB) cell number at all the exposure doses. Moreover, the irradiated mice showed a dose-dependent depletion in superoxide dismutase (SOD) activities and total anti-oxidative capacity (T-AOC), while elevation in the malondialdehyde (MDA) contents, however, gingerol treated mice after irradiation increased activities of SOD and T-AOC, and decreased the MDA levels in liver to a certain extent in all the exposure dose groups. In addition, the gingerol reduced the numbers of micronuclei (MN) of polychromatic erythrocytes (PCEs) in the bone marrow after mice were irradiated by 0, 1, 3, 5 Gy of $\gamma$-ray. Together, the findings indicated that gingerol had the therapeutic effects on mice against hematopoietic suppression and anti-oxidative damage caused by irradiation.

**Key words:** *Zingiber officinale* extract, $^{60}$Co, $\gamma$-ray, mice, free radical, superoxide dismutase (SOD), radiation; hematopoietic suppression, antioxidation.

**INTRODUCTION**

Ionizing radiation hardly damages cells and tissues because of the generation of radicals such as $\cdot$OH, $O_2^\cdot$. The ionizing radiation with low liner energy transfer can cause bone marrow suppression and depletion of blood cells in the peripheral blood. Patt et al. (1949) was the first to used cysteine to protect rats and mice against radiation-induced sickness and mortality in 1949. Since then, several chemical compounds have been reported for their radio-protective functions. However, the practical application of these synthetic chemicals especially thiols was limited because of the toxic side-effects at radio-protective dose. So, it is very important to explore the effective and nontoxic alternatives of the chemical compounds. Several cytokines such as IL-1, IL-3, IL-6, G-CSF and GM-CSF have been reported to present radio-protective effects on mice, but deleterious effects of these radio-protectors, for example, headache, diarrhea, abdominal pain and so on, has severely restrained their utility in clinics (Vincent and Gallicchio, 1988; Neta et al., 1992; Singh and Yadav, 2005).

Natural plants and their products have been studied more and more because of their easy obtaining, nontoxicity and wide acceptability. Many plants have been used to treat ailments in human beings since the advent of human history, and their preparations have been considered to be nontoxic and safe compared to synthetic...
compounds. Recently, abundant studies on plant radioprotective effects have been reported such as decreasing mortality, anti-oxidative activity, improving hematopoietic function and so on (Ohnishi et al., 1990; Sancheti and Goyal, 2007; Kumar and Kuttan, 2004; Jagetia et al., 2003; Pande, 1998; Samarth, 2004; Jagetia et al., 2004; Jegetia et al., 2006). Thus, plants and plant products can be the best choice of alternative radioprotectors.

The rhizome of *Zingiber officinale* known as ginger is widely consumed worldwide as a common spice and flavoring agent. In many countries, especially in China and India, the fresh ginger is used to prepare vegetable dishes, tea, acidophilus milk and many other food preparations. As a kind of important Chinese medicine herb, ginger and its extracts have been reported diminishing inflammation (Tsai et al., 2005; Grzanna et al., 2005), treating vomiting (Ahmed et al., 2000; Suk, 1993; Stoiolova et al., 2007; Kota et al., 2008), acting as an antitumor agent (Manju and Namasivayam, 2000; Takada et al., 2005), improving immunity (Chang et al., 2005; Pande, 1998; Samarth, 2004; Jegetia et al., 2004; Goyal, 2007; Kumar and Kuttan, 2004; Jegetia et al., 2006) and acting as a radioprotective effect agent (Jagetia et al., 2003; Jagetia et al., 2004; Du et al., 2010).

Weidner and Sigwart, (2000) showed that, a standardized ginger extract EV.EXT™ 33 at dose up to 100 mg/kg did not show any side effects on blood glucose, blood coagulation, systolic blood pressure or heart rate in rat (Weidner and Sigwart, 2000).

Gingerol, one of active ingredients in ginger, has a high anti-oxidation in vitro and in vivo experiment (Jagetia et al., 2003; Jagetia et al., 2004; Yuki et al., 2004). The medical effects of ginger were attributed to chemical substances of ginger, such as 6-gingerol, 8-gingerol, 10-gingerol, 6-zingiberene phenol, 8-zingiberene phenol and 10-zingiberene phenol (Schwertner and Rios, 2007). The immunomodulatory effects of gingerol on irradiated mice were studied in our lab, and previous study indicated that gingerol possessed the radioprotective effect on immune system in mice pre-treated with gingerol (Du et al., 2010). In this study, we evaluated the therapeutic effects of gingerol on hematopoietic system and lipid peroxidation in liver of $^{60}$Co γ-ray irradiated mice.

### MATERIALS AND METHODS

**Chemicals**

SOD kit, T-AOC kit, MDA kit, protein quantification kit and Giemsa dye kit are purchased from Jiancheng Bioengineering Institute, Nanjing, China. Fetal bovine serum (FBS) is purchased from Sigma (St. Louis, MO).

**Animals and treatment**

6~8 weeks old female Balb/c mice, 25~30 g were obtained from the Laboratory Animals Center of Sichuan University (Sichuan, China) and seventy two mice were randomly divided into 6 groups (n=12). The animals were maintained under controlled conditions of temperature (23±2°C), humidity (50±5%) and light (14 and 10 h of light and dark) respectively, and had free access to food and water. Gingerol (Xiaocao Botanical Development Co., Ltd, Xi’An, China) was dissolved in triple distilled water. Six mice of each group were irradiated with 0, 1, 3, 5, 7 Gy of $^{60}$Co-gamma-radiation at the rate of 0.8 Gy/min, and within 30 min after irradiation, they were given gingerol at 800 mg/kg B.W.

Intra-gastrically once daily for 5 days, served as radiation+gingerol groups. The 0 Gy radiation+gingerol group was considered as gingerol group. The other six mice were irradiated administrated with 0, 1, 3, 5, 7 Gy of γ-ray and given 0.5 mL double-distilled water intra-gastrically after radiation for 5 days, served as radiation+DDW groups. The 0 Gy radiation+DDW group was considered as control group.

**Peripheral blood cells number**

Animals were weighed on the day 2 after the last gavage and blood samples were collected by vena puncture with glass capillaries. Blood sample were collected to tubes disposed with ethylenediaminetetraacetic acid (EDTA)-K$_2$ beforehand and the number of blood cells including WBC, RBC, LYM and HGB were determined in 30 min.

**Relative organ weight**

Mice were sacrificed by cervical dislocation after blood was sampled. The spleen and liver were taken out, excised from adhering tissues and weighed individually. The relative organ weights were calculated according to the following equation:

$$\text{Relative organ weight} = \frac{\text{organ weight (mg)}}{\text{body weight (g)}}.$$

**Determination of superoxide dismutase (SOD), total anti-oxidative capacity (T-AOC) and malondialdehyde (MDA)**

Livers were stored at -70°C until SOD activity, T-AOC and MDA content were determined. The liver tissue homogenate was prepared with saline solution, then SOD activities, T-AOC and MDA levels in liver were determined strictly in accordance with the kit instruction.

**Determination of bone marrow micronuclei (MN)**

The number of MN of PCEs in the bone marrow was determined as described by Schmid (1973). The bone marrow was washed out from one side of femur with FBS. The cells suspension from femur bones was centrifuged at 1000 r/min and the cell pellet was resuspended. A drop of cells suspension was taken on a clean glass slide and push slices were made. The slides were fixed in methanol for 5 min after air drying at room temperature and stained with Giemsa dye. Then, MN counting was conducted under the optical microscope after immersing in dimethylbenzene for 5 min. About 1000 PCE cells per mouse were counted for the presence of MN.

**Statistical analysis**

All experiments were performed at least three times, and the results of a representative experiment are presented. All the data were expressed as mean±standard deviation (SD). The statistical
RESULTS

Relative organ weight

To evaluate whether gingerol influenced the organ weight which is an important indicator in pharmacological tests, the relative spleen and liver index were calculated on day 2 after intragastric administration. As shown in Figure 1A, γ-ray reduced significantly the relative spleen weight of mice in all radiation groups \( (p<0.01) \). But, gingerol significantly increased the spleen weight of radiated mice, especially 3 and 5 Gy groups \( (p<0.01) \). The liver weight was not sensitive to the radiation (Figure 1B). The relative liver weight is decreasing from 1 to 5 Gy but increases at 7 Gy and gingerol could significantly improve the relative liver weight at 1 and 5 Gy \( (P<0.05) \).

Peripheral blood cells number

Monocyte

As shown in Figure 2A, compared with control group,
mice showed a significant decreasing of WBC numbers after exposure to 3, 5 and 7 Gy of radiation ($p<0.01$). Gingerol could increase WBC number in radiation+gingerol groups and WBC numbers in 3 Gy radiation+gingerol group were significantly higher than that in 3 Gy radiation+DDW group ($p<0.05$). All the radiation+gingerol groups showed higher lymphocyte numbers compared to the concurrent radiation+DDW groups (Figure 2B). The data indicated that gingerol could promote the recovery of WBC and LYM number of irradiated mice.

**Red blood cell line**

To evaluate whether gingerol could increase the RBC and HGB numbers of mice suffering from different doses of γ-ray, blood samples were collected for determination of RBC and HGB numbers. As shown in Figure 3A, RBC number gradually decreased with the increasing of radiation dose. Compared to control group, RBC numbers in 3 Gy radiation+DDW group was significantly decreased ($p<0.05$) and that in 5 and 7 Gy radiation+DDW groups were extremely decreased ($p<0.01$). All the radiation+gingerol groups showed higher RBC number than the concurrent radiation+DDW groups, respectively. Gingerol could increase the HGB numbers and that in 3 Gy radiation+gingerol group ($p<0.05$) in particularly (Figure 3B). These results indicated that, gingerol could facilitate the recovery of RBC and HGB numbers of irradiated mice.

Figure 2. The cell numbers in the monocyte line. Blood was sampled from each mouse after γ-rays radiation. WBC and LYM cell number was examined immediately by hematology analyzer. A, white blood cells; B, lymphocytes; white bars, radiation+DDW group; diagonal bars, radiation+gingerol group. *$p<0.05$ and **$p<0.01$ were used between the two groups at the same dose, and #*$p<0.05$ and ##*$p<0.01$ were used between the radiation groups of 1, 3, 5, 7 Gy and control group of 0 Gy.
Activity of superoxide enzyme

To evaluate whether gingerol influences the SOD activities in liver, livers were collected to determine activities of SOD in different groups. As shown in Figure 4, SOD activities were decreased significantly after exposure to 3, 5 and 7 Gy of radiation. However, activities of SOD in gingerol treated mice were increased significantly, especially in 3 and 7 Gy radiation+gingerol group (p<0.05). These results indicated gingerol could improve the SOD activities of irradiated mice.

Total anti-oxidative capability

The total anti-oxidative capability in liver was also determined with T-AOC kits on day 2 after intragastric administration. As shown in Figure 5, obvious changes of T-AOC were not observed in 1, 3 and 5 Gy irradiation+DDW group, while there is a significant rise in 7 Gy irradiation+DDW group (p<0.01) compared to control group. Gingerol increased T-AOC level of irradiated mice, especially in 3 Gy radiation+gingerol group. These results demonstrated that gingerol could increase total anti-oxidative capability of irradiated mice.

Malondialdehyde content

To confirm if gingerol could inhibit lipid peroxidation reaction in liver, the levels of MDA in liver were determined with MDA kit on day 2 after intragastric administration. MDA contents in 0 Gy radiation+gingerol...
group were significantly lower than control group \( (p<0.01) \). All the radiation+DDW groups had significant higher MDA contents than that in control group. However, the radiation+gingerol groups, especially 3 and 5 Gy radiation+gingerol groups \( (p<0.01) \), showed lower levels of MDA compared with the concurrent radiation+DDW group. These data demonstrated that gingerol could decrease MDA content and ease liver damage induced by irradiation.

The number of bone marrow polychromatic erythrocyte (PCE) micronuclei (MN)

To study further whether gingerol caused a recovery of DNA damage induced by irradiation, the bone marrow
Figure 6. Analysis of MDA contents. The liver was excised from mice after γ-rays irradiation. The MDA content was determined by TBA method according to the specification and the absorbance was measured at 532 nm with the help of a UV-VIS double-beam spectrophotometer. The results were expressed by nmol/mg prot. White bars, radiation+DDW group; and diagonal bars, radiation+gingerol group. *p<0.05 and **p<0.01 were used between the two groups at the same dose, and #p<0.05 and ##p<0.01 were used between the radiation groups of 1,3,5,7 Gy and control group of 0 Gy.

Figure 7. Analysis of MN in bone marrow. Mice were sacrificed after γ-rays irradiation and bone marrow cells were isolated to test the number of MN by Giemsa staining. The number of MN in 1000 bone marrow PCEs was expressed in each group. White bars, radiation+DDW group; and diagonal bars, radiation+gingerol group. *p<0.05 and **p<0.01 were used between the two groups at the same dose, and #p<0.05 and ##p<0.01 were used between the radiation groups of 1, 3, 5, 7 Gy and control group of 0 Gy.

cells were isolated and MN numbers in PCE cells were determined by Giemsa staining. As showed in Figure 7, the number of MN in bone marrow of mice in 0 and 1 Gy radiation+gingerol group was significantly decreased compared to the concurrent radiation+DDW group (p<0.05), and MN numbers in 3 and 5 Gy radiation+gingerol groups were significantly lower than those in 3 and 5 Gy radiation+DDW groups, respectively. These results indicated that gingerol could facilitate the recovery of DNA damage induced by different doses of γ-ray.

The photos of bone marrow push slices
To determine whether gingerol could improve cell
morphology and proliferation that were destroyed by irradiation, the push slices of bone marrow were photographed and examined. As shown in Figure 8, there are different stages of cell division and different kinds of cell in 0, 1, 3 and 5 Gy groups (both radiation+DDW group and radiation+gingerol group), and the PCE cells with the color of pale blue-purple were clearly seen in the pictures. But, when the radiation dose increased to 7 Gy,
the proliferation of bone marrow cells in both two groups of 7 Gy was severely suppressed, which made the number of PCE cells decrease sharply. The intracellular substances were leaked out seriously. All of these made it difficult to count the MN numbers.

**DISCUSSION**

Radiation has threatened our life with the application of nuclear power, radiation therapy equipment, ray sterilization, household appliances, communicative tools and so on. Acute ionizing radiation can lead to radiation-induced hematopoietic damage and immunosuppression which result in different kinds of sickness, even death (Jagetia et al., 2003; Jagetia et al., 2002). As a kind of edible healthy food, ginger is one of people’s favorite spices in Asia and possesses several medical properties (Jagetia et al., 2003; Jagetia et al., 2004). In this study, organ weight, blood cell numbers, SOD activities and T-AOC levels in radiated mice increased after administration of gingerol, while MDA contents and MN numbers decreased.

The organ weight is an important indicator in pharmacological and toxicological experiments, which can reflect developmental levels and functions of organ. Spleen is an important hematopoietic organ, and also is the largest immune organ. Radiation could decrease spleen’s size, weight and the survival rate of splenocytes, and the function of lymphocyte proliferation response was also damaged by radiation (Du et al., 2010). In this study, gingerol can improve the relative spleen weight and significantly increase the relative spleen weight of 1, 3 and 5 Gy irradiated mice. So, we inferred that gingerol can boost the reconstruction of hematopoietic and immune function after irradiation.

Bone marrow is a major hematopoietic organ, and all original blood cells of each lineage were derived from bone marrow hematopoietic stem cells (HSC), which can continuously self-duplicate to the same rank cells with similar function, proliferate and differentiate to the subordinate cells of certain lineage (Domen et al., 2006).
Radiation could damage all the systems, organs and tissues of the whole body, and the hematopoietic tissue is the most sensitive to ray. The hematopoietic syndrome is induced by low doses of irradiation and is manifested by hematopoietic stem cell depletion and ultimately by the depletion of mature hematopoietic cells (Jagetia et al., 2003). In this study, γ-ray radiation decreased the number of RBC, HGB, WBC and LYM. The number of blood cells in radiation plus gingerol treated groups, especially in 3 Gy group was higher than radiation groups, suggesting gingerol could boost the recovery of hematopoietic function and increase blood cells numbers by reducing cells injure after radiation. Besides, WBC and LYM are more sensitive to radiation than RBC and HGB (Figures 2 and 3). The other reason is maybe that the life-span of mature RBC and HGB is longer than WBC, and a sharp drop of WBC was observed after radiation (Deng et al., 2005). In addition, lymphocytes have also infantile potential after differentiation. So, a rapid reduction in the number of lymphocytes is the earliest change of the blood cells after exposure to ionizing ray.

Ionizing radiation induces lipid peroxidation which can lead to DNA damage and cell death. The level of MDA could indirectly reflect the radiation damage degree of cells and tissues. Ginger and its extract have been reported to inhibit the induction of lipid peroxidation in vitro (Chung et al., 2003; Ahmed et al., 2000) and in vivo (Jagetia et al., 2003; Jagetia et al., 2004). In this study, all the radiation groups have significantly higher MDA contents than control group, and the administration of gingerol significantly reduced the amount of MDA compared to the concurrent radiation groups. So, it implied that gingerol inhibited the lipid peroxidation and scavenged lipid peroxide induced by radiation after whole-body exposure to γ-ray.

SOD is one of the anti-oxidative enzymes which are responsible for scavenging radiation-induced free radicals, lipid peroxide such as MDA, and thereby protected against radiation-induced sickness and mortality (Anscher et al., 2005). Total anti-oxidative capability, including anti-oxidative enzymes and non-enzymatic system, represents the body’s anti-oxidative status. The administration of gingerol enhanced activities of SOD and T-AOC levels, and the significant elevation was observed in 3 and 5 Gy gingerol treated groups compared to concurrent radiation groups (Figure 4 and 5). However, the significantly higher T-AOC, lower SOD activities and MDA contents were observed in 7 Gy radiation group compared to control group (Figure 4, 5 and 6). The reason may be that, high dose of irradiation damage the body so much that the body had to produce a great deal of anti-oxidative substances, which do not include SOD alone but many other anti-oxidants, to fight against radiation-induced oxidative injury in short time (Liang et al., 2004). As a result, radiation-induced MDA was mostly eliminated by abundant anti-oxidations. The results indicated the recovery of radiation-induced oxidative injury is the combined result of different kinds of anti-oxidative substances.

It is well known that the ionizing irradiation can directly/indirectly cause DNA damage, such as base loss, the formation of pyrimidine dimers, single- and double-strand breaks, and the free radical play an important role in DNA damage. The unrepaired or misrepaired DNA fragments form micronuclei if not be repaired correctly, the overmuch MN can result in cell death or chromosomal aberration (Salassidis et al., 1995; Kaspier et al., 2009). The significant decline of bone marrow MN numbers in radiation plus gingerol treated group may suggest the free radical scavenging ability of gingerol is related to the improvement of anti-oxidative activity and reduction of lipid peroxide (Figures 4, 5 and 6). We presumed that it may be an important reason for bigger organ weight and more blood cells in radiation plus gingerol treated groups.

In conclusion, the date we presented here demonstrated that gingerol had the therapeutic effects on γ-ray-induced hematopoietic damage by boosting DNA repair, and the elevation of anti-oxidative status, reduction of lipid peroxidation and decreasing of DNA damage may play important roles in radio-therapeutic function. However, the function of gingerol against radiation needs further investigations for a very long term in the future.

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