African Journal of Biotechnology Vol. 11(11), pp. 2609-2615, 7 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2411
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of *Zingiber officinale* extract on antioxidation and lipid peroxidation in mice after exposure to ^{60}Co-γ-ray

Xiaogang Du[1#], Yanyan Geng[1#], Zhou Huang[1#], Yanger Chen[1], Huaiyu Zhang[1], Hanmei Liu[1], zhiyu Chen[1], Zhihuan Wu[2], Tianzeng Song[3] and Xianyin Zeng[1]*

[1]Applied Biophysics and Immune Engineering Laboratory, College of Life Science, Sichuan Agricultural University, Ya'an 625014, Sichuan, China.
[2]Department of Pharmaceutical Engineering, Shijiazhuang Science and Technology Vocational College, Hebei 050000, China.
[3]Institute of Animal Science, Tibet Academy of Agricultural and Animal Husbandry Science, Lhasa 850000, china

Accepted 4 November, 2011

Zingber officinale **extract (ZOE) has been demonstrated to protect mice against radiation-induced sickness and mortality. In this study, we investigated protective ability of ZOE against anti-oxidative damage induced by non-lethal dose of ^{60}Coγ-ray. The optimum dose was selected by giving mice 0, 100, 200, 400, 800 or 1600 mg/kg body weight (B.W.) of ZOE (oral gavage) p.o. once daily for five days. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities increased, and malondialdehyde (MDA) contents decreased along with an increase in the ZOE dose of up to 800 mg/kg body weight. ZOE at dose of 800 mg/kg B.W. showed the best effect by enhancing anti-oxidative function and reducing lipid peroxidation. The activities of SOD, CAT and GSH-Px increased significantly, while MDA content decreased significantly in ZOE (800 mg/kg) + irradiation(IR) group when compared with double distilled water (DDW) + IR group (*$p<0.05$*). These results indicate that ZOE at 800 mg/kg body weight could provide protection against anti-oxidative damage in irradiated mice.**

Key words: Zingiber officinale extract, ^{60}Co, γ-ray, mice, free radical, superoxide dismutase (SOD), radiation, antioxidants.

INTRODUCTION

Radiation is widely used in medicine, health, energy, technology and other fields, and the deleterious effects attracted people's attention. It is generally considered that many radiation-induced biological effects could be attributed to the activated water reaction, which produced different kinds of free radicals, for example,·OH, H_2O_2, O_2· and so on (Patt et al., 1949). These free radicals can lead to inhibition or inactivation of anti-oxidative enzymes and other materials. Radiation could also directly damage the structure and function of the bioactive molecules in cells and tissues (Jensh, 1985), leading to bone marrow suppression and immunosuppression. There was an urgent need to explore physical and/or chemical means to protect against these harmful effects. Cysteine was reported firstly for protecting mice and rats against radiation-induced sickness (Patt et al., 1949). Since then, several chemical compounds and their analogues have been reported for their radio-protective effects. However, the toxicity of synthetic compounds at the radioprotective dose made it difficult for practical application. Several cytokines such as IL-1, IL-3, IL-6, G-CSF and GM-CSF have been screened for their radio-protection effect on mice, but their use was also limited because of the deleterious effects, such as headache, diarrhea, abdominal

*Corresponding author. E-mail: xyzeng@sicau.edu.cn. Tel: +86-835-2886136. Fax: +86-835-2886136.

#These authors contributed equally to the work.

pain and so on (Neta et al., 1992; Patchen, 1995; Neta, 1997). Thus, it is very necessary to explore the effective and nontoxic radio-protective agents.

Traditional herbs have been widely used by mankind for the treatment of various ailments since human history. There are abundant studies on herb anti-radiation effects such as antioxidant effect, decreasing mortality, improving immune function and so on (Ohnishi et al., 1990; Pande, 1998; Yuan et al., 2002; Jegetia et al., 2003; Sancheti and Goyal 2007). Recently, the herbs exact, embelin, was shown to have an effective function against ionizing radiation during the radiation therapy, and inhibits the growth of prostate cancer (Dai et al., 2011). Therefore, traditional herbs and extracts of herbs are the safest choice for alternative radio-protectors.

Zingiber officinale Roscoe is commonly used worldwide as one of the important spices and traditional herbs. The history of ginger as one clinical drug can be traced back to 2500 years ago (Shukla et al., 2007), and it has good effects on fever, vomiting, cough, diarrhea and so on. Its roots contain complicate ingredients in which polyphenol compounds (6-gingerol and shogaols) have an attractive antioxidant activity (Stoilova et al., 2007). Ginger was also reported for boosting digestion and preventing arteriosclerosis (Platel et al., 1996; Bhandari et al., 1998). In addition, many researchers have demonstrated that the ginger and ginger products have lots of pharmacological activities, include analgesic, anti-inflammation (Grazanna et al., 2005; Tsai et al., 2005), anti-tumor effect (Liu et al., 2002; Manju et al., 2005; Takada et al., 2005; Habib et al., 2008), improving immunity (Chang et al., 1995; Imanishi et al., 2004; Habib et al., 2008) and antioxidant effect (Motawi et al., 2011). Besides, US FDA has listed the ginger in “Generally Recognized as Safe” (GRAS), because ginger powder at a dose of 0.5 to 1.0 g ingested two to three times for periods ranging from three months to 2.5 years do not cause any adverse effects (Langner et al., 1998).

The wide use, the common acceptability, the innocuity and various pharmacological properties stimulated researchers to evaluate the radioprotective effect of ginger. Jegetia et al. (2003, 2004) demonstrated that *Z. officinale* extract (ZOE) could scavenge free radicals, enhance anti-oxidative status and reduce lipid peroxidation in mice, thus providing protection against radiation-induced sickness and mortality in mice. It was also reported that ZOE had regulatory effect against γ-rays-induced immunosuppression by means of protecting DNA against the radiation (Du et al., 2010). In this study, we further examined the effect of ZOE on antioxidant effect and lipid peroxidation in irradiated mice.

MATERIALS AND METHODS

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and malondialdehyde (MDA) kits were purchased from Jiancheng Bioengineering Institute, Nanjing, China. Sodium chloride, sodium bicarbonate, acetic acid and ethanol were bought from Reagent Co. (Ya’an, China).

Six to eight weeks old female Balb/c mice, 25 to 30 g, were obtained from the Laboratory Animals Center of Sichuan University (Sichuan, China). Mice were maintained under controlled conditions with temperature (23 ± 2°C), humidity (50 ± 5%), light (14 and 10 h of light and dark) and pathogen-free food and water. Anesthetic procedures and handling of animals were approved by the Animal Care and Use Committee of Sichuan Agriculture University and conducted according to the National Guidelines for the Care and Use of Laboratory Animals in China (Approval No: 09ZA072) .

Selection of optimum dose

ZOE (Xiaocao Botanical Development Co. Ltd., Xi’an, China), which was extracted from dry powder by ethanol and tested by high liquid performance chromatography (HPLC), was dissolved in DDW. 60 mice were randomly divided into six groups (n = 10). The optimum dose of ginger was selected by giving the animals 100, 200, 400, 800 or 1600 mg/kg B.W. ZOE p.o. once daily for five consecutive days. Mice were given 0.02 ml/g body weight (B.W.) of ZOE or DDW p.o. Mice given DDW p.o., served as control group.

Effect of ZOE on anti-oxidation of irradiated mice

To evaluate the radioprotective ability of ZOE, 20 mice were divided into two groups with ten mice per treatments: (1) DDW + irradiation group received 0.02 ml/g B.W. of DDW p.o. before irradiation; and (2) ZOE + irradiation group was given 800 mg/kg B.W. of ZOE once daily for five consecutive days before irradiation.

Irradiation

Each mouse, in a wooden container, was put in the ^{60}Co-γ irradiator (Model 220, Sichuan Academy of Agricultural Science, China), and irradiated with five Gy at the dose rate of 0.8 Gy/min. The dosimetry was carried out with Baldwin Farmer secondary dosimeter and Fricke dosimeter.

Determination of activities of SOD, CAT, GSH-Px and MDA contents

Blood sample were collected on day seven for the determination of the activities of SOD, CAT, GSH-Px and MDA contents. The determination of anti-oxidative function was performed according to the kit introduction.

Statistical analysis

All the data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted by using Prism 5.0 statistical software. The significance between the treatments was evaluated by ANOVA. Differences were considered statistically significant with $P < 0.05$.

RESULTS

Activity of superoxide enzyme

To evaluate whether gingerol influenced the SOD activities

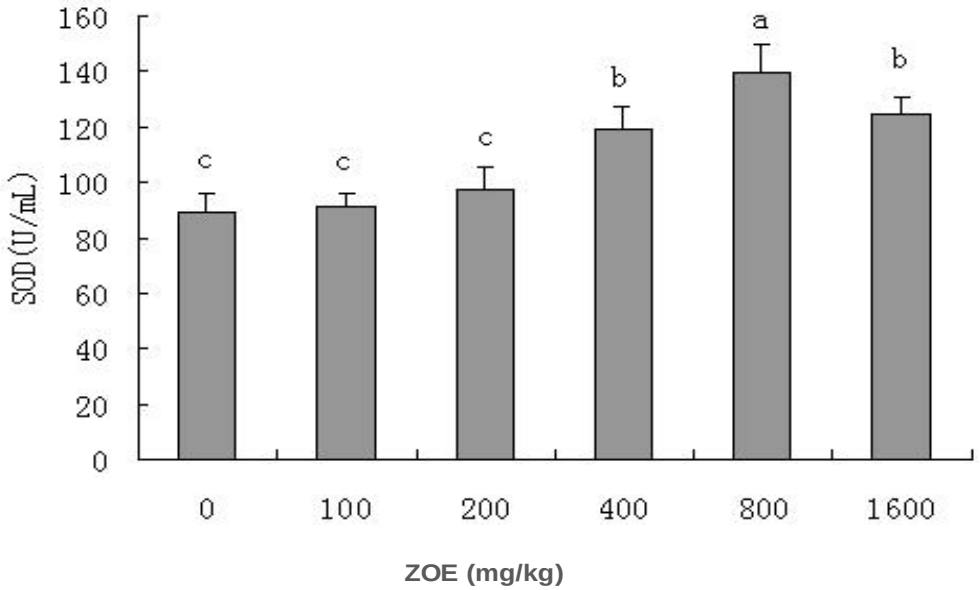

Figure 1. SOD activities of ZOE treated mice. Blood samples were collected on day seven after administration of ZOE at the doses of 0, 100, 200, 400, 800 and 1600 mg/kg B.W. for five consecutive days, and SOD activities were tested by xanthine oxidase method according to the kit specification. Means without a common letter differ at $p<0.05$.

in blood, blood samples were collected for determination of activities of SOD in different dose of ZOE treated groups. As shown in Figure 1, SOD activities in 400, 800 and 1600 mg/kg of ZOE treated groups were significantly higher than that in control group *(p<0.05)*, and 800 mg/kg of ZOE treated group showed the highest SOD activities in blood. These indicate that ZOE at dose of 800 mg/kg B.W. had the best effect on increasing SOD activities in blood of mice.

Activity of catalase

To determine whether ZOE could increase activities of CAT in blood, mice were bled for blood samples after administration of different doses of ZOE once daily for five days. As shown in Figure 2, 200, 400, 800 and 1600 mg/kg B.W. of ZOE treated groups showed significantly higher CAT activities when compared with the control group*(p<0.05)*. 800 mg/kg B.W. of ZOE treated group showed the best effect on increasing CAT activities. These results demonstrate that ZOE at the dose of 800 mg/kg had the best effect by promoting CAT synthesis and increasing CAT activities in blood.

Activity of glutathione peroxidase

To evaluate whether ZOE could increase GSH-Px activities in blood, mice were bled for blood samples on day seven after administration of different doses of ZOE. As shown in Figure 3, GSH-Px activities in ZOE treated group increased along with an increase in ZOE dose, and GSH-Px activity in 800 mg/kg B.W. was significantly higher than that in the control group *(p<0.05)*. The results indicate that 800 mg/kg B.W. of ZOE had the best effect by increasing GSH-Px activities in the blood of mice.

Contents of malondialdehyde

To determine whether ZOE could decrease lipid peroxidation, blood samples were collected from mice on day seven to determine the levels of MDA in blood. As shown in Figure 4, MDA contents decreased along with an increase in the ZOE dose of up to 800 mg/kg body weight but, 1600 mg/kg B.W. of ZOE treated group did not show a better effect on reducing MDA content in blood when compared with other dose of ZOE treated group (Figure 2). These results demonstrate that ZOE of 800 mg/kg B.W. showed the best effect by eliminating lipid peroxides.

Radio-protective effect of ZOE

To determine whether ZOE could improve anti-oxidative function of irradiated mice, blood was collected for the determination of SOD, CAT, GSH-PX activities and MDA contents. As shown in Table 1, activities of SOD, CAT and GSH-Px were significantly higher in ZOE + IR group than in DDW + IR group (*p<0.05*), while MDA content decreased significantly in ZOE + IR group when compared with DDW + IR group (*p<0.05*). The results indicate that

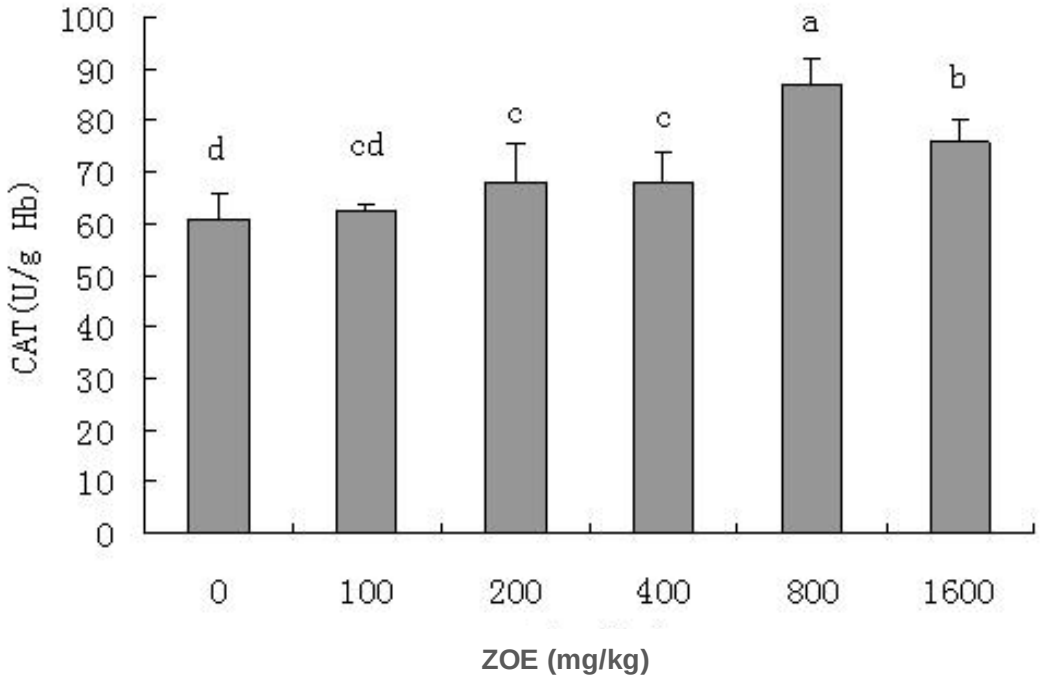

Figure 2. CAT activities of ZOE treated mice. Blood samples were collected on day seven after administration of ZOE at the doses of 0, 100, 200, 400, 800 and 1600 mg/kg B.W. for five consecutive days, and CAT activities were tested with spectrophotometry according to the kit specification. Means without a common letter differ at $p<0.05$.

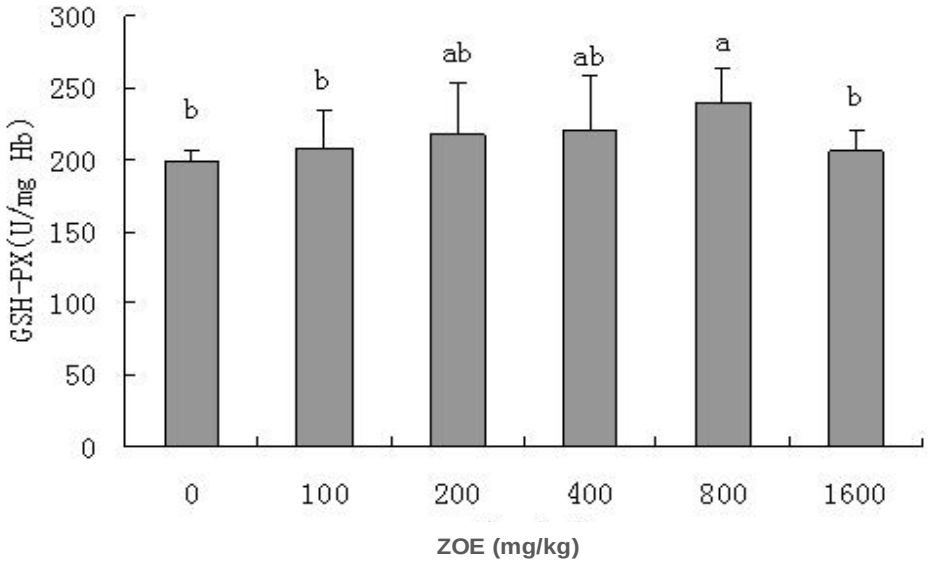

Figure 3. GSH-Px activities of ZOE treated mice. Blood samples were collected on day seven after administration of ZOE at the doses of 0, 100, 200, 400, 800 and 1600 mg/kg B.W. for five consecutive days. GSH-Px activities were determined with spectrophotometry according to the kit specification. Means without a common letter differ at $p<0.05$.

administration of ZOE before irradiation could enhance anti-oxidative function, thus providing protection against radiation-induced decrease in anti-oxidative ability and increase in lipid peroxidation.

DISCUSSION

Z. officinale roscoe is commonly used as one important spice in our daily life. Ginger and ginger extract have been

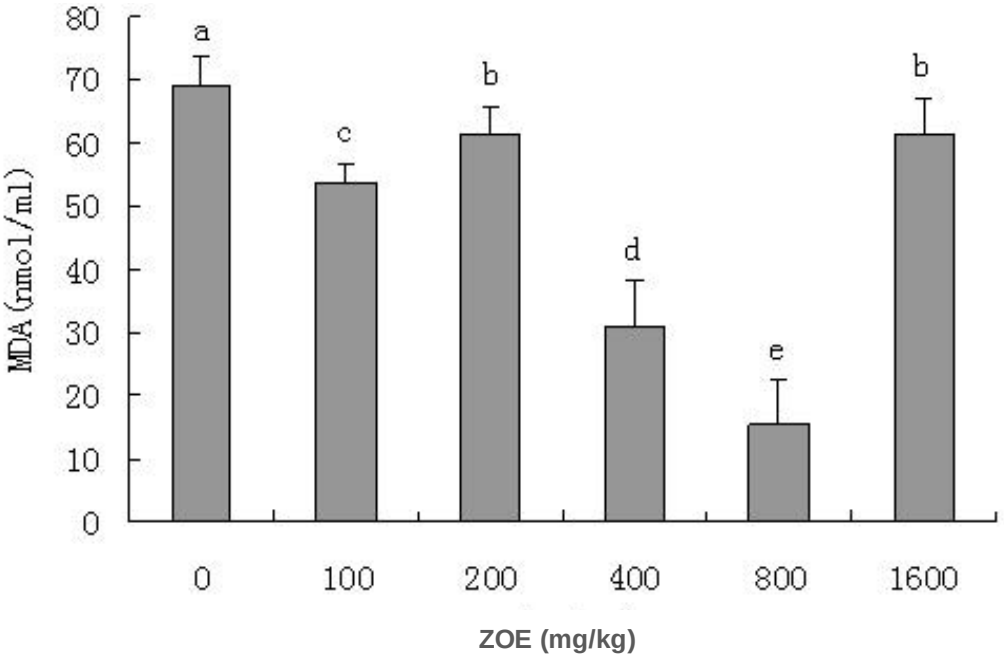

Figure 4. MDA contents of ZOE treated mice. Mice were sampled for blood samples on day seven after administration of ZOE at the doses of 0, 100, 200, 400, 800 and 1600 mg/kg B.W. for five consecutive days, and MDA contents were determined by TBA method according to the kit specifications. Means without a common letter differ at $p<0.05$.

Table 1. SOD, CAT, GSH-Pxactivities and MDA content (mean ± SD) in blood of mice treated with 800 mg/kg B.W. of ZOE once daily for five days before exposure to 5 Gy γ-radiation

Group	SOD (U/ml)	CAT (U/g Hb)	GSH-PX (U/mg Hb)	MDA (nmol/ml)
DDW + IR group	57.70±10.36	35.16±8.14	139.34±5.11	82.10±4.31
ZOE + IR group	115.78±9.99[a]	54.44±8.40[a]	167.33±14.06[a]	31.73±7.74[a]

n = 10 for each group; IR, irradiation: ZOE + IR group when compared with DDW + IR group; [a] p<0.05.

reported for their pharmacological activities. There are also several reports showing that *Z. officinale* extracts (ZOE) can enhance anti-oxidative function and decrease lipid peroxides against irradiation at the lethal dose (Jegetia et al., 2003, 2004). However, few reports are known about the effects of ginger extract on anti-oxidative function of the irradiated mice exposed to non-lethal dose of γ-ray. In this study, we demonstrate that ZOE can promote anti-oxidative ability in 5 Gy of γ-ray-irradiated mice.

O_2 is reduced to H_2O after receiving four electrons under the normal physiological condition, but partial reduction of O_2 induces the highly reactive oxygen species (ROS), including the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$). ROS can attack the bioactive molecules such as DNA protein and lipids, and damage their structures and functions, leading to aberrant downstream signaling or stimulation of apoptosis (Finkel, 1998; Thannickal et al, 2000). SOD, CAT and GSH-Px are important anti-oxidative enzymes which can scavenge the superfluous free radicals, thereby protecting cells from damage and decreasing the deleterious species or lipid peroxides, such as MDA (Curello et al., 1987; Anscher et al., 2005). In this study, the content of the antioxidant enzymes such as SOD, CAT and GSH-PX increased in a dose-dependent pattern up to 800 mg/kg body weight after mice were treated with ZOE, but content of these antioxidant enzymes increased above 800 mg/Kg body weight (Figures 1, 2 and 3). It suggests that ZOE has the strong anti-oxidative effect, and the antioxidant effect is dose-dependent.

MDA is an important oxidative metabolite of polyunsaturated fatty acids, which consists of the biomembrane (Buege et al, 1978). MDA is often seen as an indicator of the oxidation status in cells or tissues. So, the high level of MDA is detrimental to cells and tissues, and leads to lose of their normal bio-function (Cheng et al, 2011). In this study, MDA content was the lowest in 800 mg/kg ZOE treated group, and it decreased significantly when compared with control group (Figure 4). However, MDA

content increased in 1600 mg/kg ZOE treated group. These results suggest that ZOE at a proper dose showed strong anti-oxidative ability. This is in agreement with the research of Motawi et al. (2011) which reported that ginger exact is an attractive candidate for the treatment of liver fibrosis induced by CCl_4 by down regulating free radicals elevation and enhancing hepatic antioxidant levels. It implied that ZOE at the optimum dose of 800 mg/kg may effectively scavenge the excessive free radicals and other deleterious substances.

Radiation is a factor that produces free radicals, and can change the normal structure and function of cells and tissues. Finally, the radiation leads to the radiation disease (Jensh, 1985). In this study, the administration of ZOE (800 mg/kg body weight) after the radiation enhanced the activities of SOD, CAT and GSH-Px when compared with the concurrent DDW + IR group, but MDA content was significantly decreased (Table 1). So, it implied that 800 mg/kg body weight of ZOE can improve anti-oxidative damage and inhibit the lipid peroxidation caused by irradiation, thereby protecting against radiation-induced sickness and mortality (Anscher et al., 2005).

In summary, the data presented here clearly indicate that ZOE at 800 mg/kg body weight had radioprotection against radiation-induced antioxidant damage by scavenging the excessive free radicals and lipid peroxides caused by irradiation. However, the specific mechanism of ZOE in enhancing antioxidant activity needs further investigation in the future.

ACKNOWLEDGEMENTS

This work was supported by the program for Changing Scholars and Innovative Research Team in University (IRT0848), and Sichuan Education Commission (Project No. 09ZA072). We also thank the Institute of Biotechnology and Nuclear-technology, Sichuan Academy of Agricultural Science, for providing the necessary irradiation facilities.

REFERENCES

Anscher MS, Chen L, Rabbani Z, Kang S, Larrier N, Huang H, Samulski TV, Dewhirst MW, Brizel DM, Folz RJ, Vujaskovic Z (2005). Recent progress in defining mechanisms and potential targets for prevention of normal tissue injury after radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. 62(1): 255-259.

Bhandari U, Sharma JN, Zafar R (1998). The protective action of ethanolic ginger(Zingiber Officinale) extract in cholesterol fed rabits. J. Ethnopharmacol. 61(2): 167-171.

Buege JA, Aust SD (1978). Microsomal lipid peroxidation. Meth. Enzymol. 52: 302-310

Chang CP, Chang, Wang JY, Wang FY, Chang JG (1995). The effect of Chinese medicinal herb Zingiberis rhizoma extract on cytokine secretion by human peripheral blood mononuclear cells. J. Ethnopharmacol. 48(1): 13-19.

Cheng J, Wang F, Yu DF, Wu PF, Chen JG (2011). The cytotoxic mechanism of malondialdehyde and protective effect of carnosine via protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons. Eur. J. Pharmacol. 650(1): 184-194.

Curello S, Ceconi C, Cargnonl A, Cornacchiarl A, Ferrari R, Albertini A (1987). Improved procedure for determining Glutathione in plasma as an index of Myocardial oxidative stress. Clin. Chem. 33(8): 1448-1449.

Dai Y, Desano J, Qu Y, Tang W, Meng Y, Lawrence TS, Xu L(2011). Natural IAP inhibitor Embelin enhances therapeutic efficacy of ionizing radiation in prostate cancer. Am. J. Cancer. Res. 1(2): 128-43.

Du X, Pan H, Zhang C, Zhang H, Liu H, Chen Z, Zeng X (2010). Zingiber officinale extract modulates γ-rays-induced immunosuppression in mice. J. Med. Plants. Res. 4(16): 1647-1655.

Finkel T (1998). Oxygen radicals and signaling. Curr. Opin. Cell. Biol. 10(2): 248–253.

Grazanna R, Lindmark L, Frondaza CG (2005). Ginger-An Herbal Medicinal Product with Broad Anti- Inflammatory Actions. J. Med. Food. 8(2): 125-132.

Habib SHM, Makpol S, Hamid NAA, Das S, Ngah WZW, Yusof YAM (2008). Ginger extract (Zingiber officinale) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. Clinics. 63(6): 807-813.

Imanishi N, Mantani N, Sakai S, Sato M, Katada Y, Ueda K, Terasawa K, Ochiai H (2004). Inducible Activity of Ginger Rhizome (Zingiber Offifinale Rosc.) on the mRNA Expression of Macrophage-Inducible Nitric Oxide (NO) Synthase and NO Production in a Macrophage Cell Line, RAW264.7 Cells. Am. J. Chinese. Med. 32(5): 727-735.

Jagetia GC, Baliga MS, Aruna R, Rajanikant GK, Jain V (2003). Effect of abana (a herbal preparation) on the radiation-induced mortality in mice. J. Ethnopharmacol. 86: 159-165.

Jagetia GC, Baliga MS, Venkatesh P (2004). Ginger (Zingiber officinale Rosc.), a Dietary Supplement, Protects Mice Against Radiation-Induced Lethality: Mechanism of Action. Cancer. Biother. Radio. 19(4): 547-561.

Jagetia GC, Baliga MS, Venkatesh P, Ulloor JN (2003). Influence of ginger rhizome [Zingiber officinale Rosc] on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. Radiat. Res. 1(160): 584–592.

Jensh RP (1985). Ionizing radiation and the conceptus: neurophysiologic effects of prenatal X-radiation on offspring. Ann. Clin. Lab. Sci. 15(3): 185-194.

Langner E, Greifenberg S, Gruenwald J (1998). Ginger: history and use. Adv. Ther. 15: 25-30.

Liu H, Zhu Y (2002). Effect of alcohol extract of Zingiber officinale rose on immunological function of mice with tumor. Wei Sheng Yan Jiu. 31(3): 208-209.

Manju V, Nalini N (2005). Chemopreventive efficacy of ginger, a naturally ocurring anticarcinogen during the initiation, post-initiation stages of 1, 2 dimethylhydrazine induced colon cancer. Clin. Chimica Acta. 358: 60-67.

Motawi TK, Hamed MA, Shabana MH, Hashem RM, Aboul Naser AF (2011). Zingiber officinale acts as a nutraceutical agent against liver fibrosis. Nutr. Metab. 8:40.

Neta R (1997). Modulation of radiation damage by cytokines. Stem Cells. 15(2): 87-94.

Neta R, Peristein R, Vogel SN, Ledney GD, Abrams J (1992). Role of interleukin 6(IL-6) in protection from lethal irradiation and in endocrine responses to IL-1 and tumor necrosis factor. J. Exp. Med. 175(3):689-694.

Ohnishi Y, Yasumizu R, Fan HX, Liu J, Takao-Liu F, Komatsu Y, Hosoya E, Good RA, Lkehara S (1990). Effects of juzen-taiho-toh (TJ-48), a traditional Oriental medicine, on hematopoietic recovery from radiation injury in mice. Exp. Hematol. 18(1): 18-22.

Pande S (1998). Evaluation of radiomodifying effects of root extract of panax ginseng. Phytother. Res. 12: 13-17.

Patchen ML (1995). Amifostine plus granulocyte colony-stimulating factor therapy enhances recovery from supraletal radiation exposures: preclinical experience in animals models. Eur. J. Cancer. 31(1): S17-21.

Patt HM, Tyree EB, Straube RL, Smith DE (1949). Cysteine protection against X-irradiation. Science, 110(2852): 213-214.

Platel K, Srinivasan K (1996). Influence of dietary spices on their active

principles on digestive enzymes of small intestinal mucosa in rats. Int. J. Food Sci. Nutr. 47(1): 55-59.
Sancheti GK, Goyal P (2007). Prevention of radiation induced hematological alteration by medicinal plant Rosmarinus officinalis in mice. Afr. J. Trad. Cam. 4(2): 165-172.
Shukla Y, Singh M (2007). Cancer preventive properties of ginger: A brief review. Food. Chem. Toxicol. 45: 683-690.
Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food. Chem. 102: 764-770.
Takada Y, Murakami A, Aggarwal BB (2005). Zerumbone abolishes NF-kappaB and IkappaBalpha kinase activation leading to suppression of antiapoptotic and metastatic gene expression, upregulation of apoptosis, and downregulation of invasion. Oncogene, 24(46): 6957-69.
Thannickal VJ, Fanburg BL (2000). Reactive oxygen speciesin cell signaling. Am. J. Physiol. 279(6): 1005-1028.
Tsai TH, Tsai PJ, Ho SC (2005). Antioxidant and Anti-inflammatory Activities of Several Commonly Used Spices. JFS C: Food. Chem. Toxicol. 70(1): 93-97.
Yuan D, Sunouchi H, Sakurai T, Saito K, Kano Y (2002). Pharmacological properties of traditional medicines interaction between Ephedra Herb and Gypsum under hyperthermal conditions in rats. Biol. Pharm. Bull. 25(7): 872-874.