Protective effects of sulfated derivatives of polysaccharides extracted from *Auricularia auricular* on hematologic injury induced by radiation

Z. Hua, W. Zhen-Yu*, Y. Xin, Y. Lin, Z. Li-Li, B. Hai-Na, L. Xiao-Yu

School of Food Science and Engineering, Harbin Institute of Technology, 73 HuangHe Road, NanGang District, Harbin, 150090, P. R. China

► ***Original article***

**** Corresponding author:***
Prof. Wang Zhen-Yu,
Fax: *+86 451 86282909*
E-mail:
wzy219001@yahoo.com.cn

Received: Jan. 2013
Accepted: Apr. 2013

Int. J. Radiat. Res., April 2014; 12(2): 99-111

ABSTRACT

***Background*:** Ionizing radiation induces the production of reactive oxygen species (ROS), which play an important causative role in cell death. The aim of this study was to investigate the protective effects of sulfated derivatives of neutral polysaccharides extracted from Auricularia auricular (SNAAP). ***Materials and Methods*:** Whole blood samples from healthy donors treated with SNAAP at different concentrations (20, 60, 100 µg/mL) were exposed to various doses of X-rays. Wistar rat spleen lymphocytes, in cultures, were treated with SNAAP at different concentrations (20, 60, 100 µg/mL) in the presence p.o 12 hours prior to 8 Gy gamma radiation exposure. Animals were administered with SNAAP at doses of 50, 100 or 200 mg/kg body weight d p.o 7 days prior to sub-lethal doses (6 Gy) of whole body gamma radiation exposure. ***Results*:** SNAAP is an effective radio protector against X-ray radiation induced in vitro cellular damage in human peripheral blood. Furthermore, to support this finding the effect of SNAAP on a rat's spleen lymphocytes, when cultured and examined 24 hours after exposure to 8 Gy γ of radiation, demonstrated the effect of polysaccharides on a rat's spleen lymphocytes, pretreated by the SNAAP, can increase the cell viability compared with irradiated group at a concentration of 20, 60 and 100 µg/mL. Likewise, this radiation-induced therapy decreased each mouse's body weight and effectively stimulated the immune system of all radiated mice. Moreover, when induced by Co60, the SNAAP decreased the level of malondialdehyde (MDA) and increased the myeloperoxidase (MPO) and the glutathione peroxidase (GSH-Px) activity in the whole blood supply of the irradiated mice. ***Conclusion*:** These encouraging results support further research into the clinical pharmacology of SNAAP as a novel agent for human radiation protection.

Keywords: *Polysaccharides, auricularia auricular, radioprotection, sulfated.*

INTRODUCTION

Humans are constantly exposed to ionizing radiation from natural sources such as cosmic rays experienced during space travel and again by radioisotopes found in the earth's crust and then again from a wide variety of artificial sources [1]. Gamma-ray radiation is known to induce oxidative stress via the generation of reactive oxygen species in cells [2]. In many cases, radiation-induced cell death has been identified as Apoptosis [3, 4]. Moreover, the human hematopoietic system is highly sensitive to ionizing radiation. Likewise, there is general acknowledgement that radiation, as chemotherapy, destroys the hematopoietic stem and progenitor cells resulting in rapid loss of

peripheral blood cells. The damage caused by this loss applies particularly to leukocytes needed as a host defense against microbial invasion.

Lymphocytes have been used to develop non-invasive bioassays to screen humans for toxicant exposure; these cells have been used to determine exposure and susceptibility to toxicants [5-7]. In an early study on the effect of radiation on human leukocytes; Sokolov (1961) found by means of an electron microscope study of X-ray damaged frog blood cells, when measured one day after irradiation; that an X-ray dose of 90 Gy caused a 30% drop in the leukocyte count [8].

Furthermore, and in a more recent study, Williams *et al.* (2010) found that by decreasing the peripheral lymphocyte count over the first two or three days of treatment that this practice is a reliable indicator of exposure to humans. Similarly, with a radiation dose as low as 0.5 Gy a 50% decrease of a peripheral lymphocyte occurred within 24 hours and further decreased over 48 hours to bring about a lethal exposure [9].

In relation to radiation damage in humans, it is important to search possible radio-protective agents to modify the normal response of biological systems to radiation-induced toxicity or lethality [10]. Of late, scholars have researched many natural and synthetic chemicals for their efficacy to protect against radiation-induced damage in biological systems. However, the inherent toxicity of some of the synthetic agents, at an effective radio-protective concentration, warrants a further evaluation for safer and more effective radio-protectors [11]. In this respect, natural antioxidants, which have a low potential for side effects, while maintaining efficacy in physiologic concentrations, demonstrate potential as protectors [12, 13]. Likewise, crude extracts from medicinal herbs and their preparation have constituted several effective radio-protective drugs [14].

Over the past three decades, and in tandem with this research, many polysaccharides and polysaccharide-protein complexes have been isolated from mushrooms, fungi, yeasts, algae, lichens and plants. Due to their immuno-modulatory and anti-tumor effects, the biological activities of polysaccharides have gained attention in the biochemical and medical disciplines [15]. For example, in a recent study, Li and Zhou [16] in research dissimilar to this study, found the oral administration of dose dependent polysaccharides taken from the berry of the Chinese ginseng bush (*cì wǔ jiā* (刺五加): *Acanthopanax senticosus*); reduced the irradiation-induced injury on rats and imparted a protective effect against 15 Gy X-ray irradiation-induced loss of BW, WC, food and water intake. Moreover, it reduced the rat's MDA level and raised their anti-oxidase activity (SOD, GSH-Px).

The *Auricularia auricular* mushroom species is the fourth most important cultivated mushroom used by humans throughout the world that offer polysaccharides and polysaccharide-protein complexes [17]. A review of available research literature found many published studies have examined the beneficial health benefit from *Auricularia auricular* on both humans and animals. Of significance, *Auricularia auricular* is known for its pharmaceutical effects, for example, the hindering of lipid per-oxidation and subsequent decreased liver damage [18, 19]. *Auricularia auricular* is also known as a scavenging radical and for chelating metal ions in vitro [19] it is also known as an anti-oxidant and known , as well, for its hypolipidemic properties [20]. In a more recent study, Wu *et al.* [21] found that *Auricularia auricular* has a pharmacological effect on the left ventricle ejection fraction in aged mice.

By using various established in vitro systems, the sulfation of neutral polysaccharides extracted from *Auricularia auricular* (SNAAP) and its antioxidant activities were investigated by Zhang et al. [22] The results clearly showed that the antioxidant activity of sulphated polysaccharides extracted from *Auricularia auricular* were signiDcantly improved related to the ability of scavenging superoxide radicals. Due to the formation of oxygen radicals, the radiation effect on the human body results in oxidative stress. To ensure successful radiation protection, it is possible to control damage due to radiation by antioxidants and by

anti-oxidative enzymes. Nonetheless, owing to the radiation treatment and the practical radiation dose, when applied in the radiation protection process, it is important to indicate the relationship between the treatment of antioxidants and the oxidative damage of biomolecules in both the target and non-target tissues.

In the realm of medical safety the hazard of radiation exposure tenders not only an enormous problem but also offers a biological safety challenge for human beings; especially when caused by water radiolysis and the deleterious effects of ionizing radiation in biological systems [23]. This study, using polysaccharides and polysaccharide-protein complexes found in the *Auricularia auricular* mushroom species, proposes a method as a protector against this challenging problem. Therefore, the paper's purpose explores the effect of the sulfation of neutral polysaccharides radioprotection X-ray radiation on human blood and blood components and of the radioprotection γ-ray radiation on the viability of spleen lymphocytes.

The methods employed in this study firstly evaluate the X-ray radioprotection of SNAAP on human peripheral blood using the manual white blood cell counting (WBC) technique that incorporate different concentrations of sulfated neutral polysaccharides (20, 60, 100 μg/mL). A second examines the γ-ray radioprotection of SNAAP in cultured Wistar rat spleen lymphocytes. A third examines the γ-ray radioprotection of SNAAP in 50 laboratory mice, which includes a biochemistry measurement. The results conclude that the pre-treated irradiated human blood samples showed a decrease in the total white blood cell count. Furthermore, when pre-treated with a 60 μg/mL concentration of SNAAP, when followed by 12 hours exposure to X-ray radiation, increased the white blood cell count and improved the morphological changes of the white blood cell classification.

When considering the preceding problem, and as a means to offer radioprotection for X-ray radiation on human blood and blood components, the use of the antioxidant activities found in SNAAP as an effective radio protector against X-ray radiation and as a novel agent for human radiation protection is explored in this research.

MATERIALS AND METHODS

Samples

The *Auricularia auricular* sample originated from Heilongjiang Province, PR China. The peripheral blood samples were aseptically collected in $EDTAK_2$ sterile glass tubes from the median cubital vein of a non-smoking healthy female Chinese donor approximately 25–35 years of age and in accordance with China's blood specimen collection and ethical processing regulations. Written consent was obtained from the participant. The adult male Wistar rats and 50 Kunming mice of equal sex were purchased from the Laboratory Animal Center of the Second Hospital of the Harbin University, Heilongjiang Province, P R China.

Reagents and Apparatus

Trifluoroacetic acid (TFA) was purchased from E. Merck, Darmstadt, Germany. D-mannose, L-rhamnose, D-ribose, D-glucose, D-xylose, D-galactose, and L-arabinose were all purchased from Sigma St. Louis, USA, and the T-series Dextrans from Agilent, Beijing, China. Superoxide Dismutase (SOD), Malondialdehyde (MDA) and the Glutathione Peroxidase (GSH-px) commercial kits for detecting enzyme activation were all purchased from Jiancheng Institute of Biotechnology, Nanjing, China. Agilent 6890N Gas Chromatograph/5973 Mass-Selective detector was purchased from Agilent Co., Ltd. USA. The microscope (XSZ-H) was purchased from Fei Ji Technology Co., Ltd. Nanjing, China.

The preparation of SNAAP and A composition analysis of the Monosaccharide (GC/MS)

The SNAAP was prepared as described by Zhang [22]. Gas chromatography Mass-Selective detector (GC/MS) was used for further identification and quantification of the monosaccharides. The SNAAP were hydrolyzed

for 4 hours with 2 mol/L TFA at 110˚C[24]. The SNAAP's monosaccharide's were conventionally converted into the alditol acetates as described Oades and Johnes and Albersheim with a slight modification and then analyzed by an Agilent 6890N Gas Chromatograph/5973 Mass-Selective detector equipped with a capillary column—DB-5, 60 m × 0.25 mm i.d. × 0.25 μm [25, 26]. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injection volume was 1 μL and split ratio was 1:10. The temperature program was set to increase from an initial 200˚C to 250˚C at 25˚C min^{-1} and held for ten minutes. The inlet temperature was kept constant at 250˚C and the MS transfer line was set at 250˚C. The ion source and quadrupole temperature were respectively: 150˚C and 230˚C. The Mass Spectrophotometer (MS) acquisition parameters included scanning from m/z 35–450 in the electron impact (EI) mode for routine analysis.

The evaluation of X-ray radioprotection of SNAAP on human peripheral blood

Experiment Design [27]

The human peripheral blood was divided into five groups:

Group I: Peripheral blood.

Group II: X-ray-irradiated Peripheral blood.

Group III: X-ray + Peripheral blood pretreatment with of SNAAP (20 μg/mL).

Group IV: X-ray + Peripheral blood pretreatment with of SNAAP (60 μg/mL).

Group V: X-ray + Peripheral blood pretreatment with of SNAAP (100 μg/mL).

Radiation Exposure

Whole-blood cultures were supplemented with 20, 60, and 100 μg/mL by a final concentration of SNAAP 2 hours prior to receiving the X-ray dose of radiation (30 Kv 5mA–20 minutes) with a radiation field of 100mm × 100mm and a radiation length of 20 cm. Cultures in plastic dishes were kept at 37°C in a humidified 5% CO_2 atmosphere. For counting purposes, the white blood cells (WBC) were isolated from the whole blood 12 hours post-radiation.

Effect on WBC in Human Peripheral Blood

Whole venous human blood well mixed and anti-coagulated ($EDTAK_2$) was diluted with a 3% acetic acid solution that hemolyzed mature erythrocytes and facilitated leukocyte counting. The standard dilution for a leukocyte count is 1:20. The cells were allowed to settle and then were counted in specific areas of the hemacytometer chamber under the microscope [28].

Effect on morphology in human peripheral white blood cells

Blood films were a simple procedure[29,30] and made by placing a drop of human blood on one end of a glass slide and using a spreader slide to disperse the blood over the slide's length. Each slide was left to air dry after which the blood was secured to the slide by immersing it briefly in methanol. Securing the blood to the slide in such a manner is essential for good staining and presentation of cellular detail. Following this procedure, the slide was stained with blood film to distinguish the cells from each other.

Radio-protective effects of SNAAP on rat spleen lymphocytes irradiated *in-vitro* by gamma-rays

Preparation of spleen lymphocytes

The spleen of an adult male Wistar rat was finely chopped and placed in a sterile Hank's Balanced Salt Solution (HBSS). The spleen lymphocytes cells were then expressed through a stainless steel gauze. The remnant tissues depleted of the single cells were kept separately. The cell suspensions were centrifuged at 560 × g for ten minutes and the lymphocytes were then harvested. To eliminate red blood cells, the recovered cells were treated for 5 minutes with an ammonium chloride/EDTA solution (0.83% NH_4Cl, 0.1% $KHCO_3$, 0.37% EDTA, pH 7.4) at 4 °C and were then washed twice with phosphate-buffered saline [31]. Preparation of Spleen Lymphocytes were repeated three times. The viability of the obtained cells was approximately 95% when measured by the Trypan Blue Dye Exclusion test each time.

Experiment Design

The Wistar rat's spleen lymphocytes were divided into five groups:

Group I: Control Lymphocytes.

Group II: Gamma Lymphocytes.

Group III: Gamma Lymphocytes pretreatment with of SNAAP (20 μg/mL).

Group IV: Gamma Lymphocytes pretreatment with of SNAAP (60 μg/mL).

Group V: Gamma Lymphocytes pretreatment with of SNAAP (100 μg/mL).

Description of cell line radiation

Prior to radiation, and to achieve the cell synchronization, the cells were grown to 70-80% confluence and placed in 1% serum-containing media for two hours. Cell monolayers were pretreated with SNAAP (20-100 μg/mL) at 37 ℃ for 12 hours and then irradiated with single dose (8 Gy) of γ-ray at a dose rate of 1.59 Gy/min using a Co^{60} irradiator (Maize Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin, China). After radiation, the cells were incubated and kept for up to 24 hours in a 10 % serum containing media in a 5% CO_2 atmosphere at 37℃.

In-vitro Cell viability analysis

The effect of the SNAAP on the viability of the rat's spleen lymphocytes was determined based on the reduction of a Tetrazolium salt by mitochondrial dehydrogenase in viable cells [32]. The spleen lymphocytes were treated with the SNAAP at 20-100 μg/mL and with γ-radiation. Twenty hours later, 10 μL of the MTT stock solution (2 mg/mL) was added to each well to reach a total reaction volume of 110 μL. Incubating for another 4 hours, the plate was centrifuged at 2200 × g for 5 minutes followed by aspiration of the supernatants. Formazan crystals in each well were dissolved in 100 μL of DMSO and an A_{490} reading was read on a scanning multi-well spectrophotometer.

Radio-protective effects of SNAAP against radiation-induced oxidative injury to mice

All mice were kept under standard conditions of temperature and humidity in the Centre's Animal House Facility and provided with standard mouse food (purchased from the Laboratory Animal Center of the Second Hospital of the Harbin University, Harbin, China) and water ad libitum. All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee, strictly adhering to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals constituted by Experimental Animal Management Ordinance of China.

Radiation Exposure

Radiation was conducted by the method adopted by the Maize Research Institute of Heilongjiang Academy of Agricultural Sciences. The mice from each irradiated group were bodily irradiated for 6.15 minutes with a single 6 Gy dose delivered by a Co^{60} tube operated with an output of 1.01 Gy/min at 160 cm.

Polysaccharides treatment

The SNAAP, of varying doses (50, 100, 200 mg/kg.d), was dissolved in sterile saline and was orally given to the mice a week before radiation on the basis of our experience. The control mice were orally given the same volume of sterile saline.

Effect of SNAAP on radiation-induced change of mice body weight and biochemistry measurement

Before receiving radiation, the body weight of each mouse was measured daily. Measurements at 37 ℃ by 0.8~1.2 mL of blood sample taken on the first day after receiving radiation under standard assay conditions were implemented by an automatic analyzer (Sysmex F-800 Hematology Analyzer, Japan) and in accordance with the Randox application procedure. Malondialdehyde (MDA) level, myeloperoxidase (MPO) activity, and Glutathione Peroxidase (GSH-Px) activity in the whole blood of irradiated mice were measured in accordance with the commercial kit manufacturer's instructions using commercially available kits (Nanjing Jiancheng, China) [33]. All the above treatments were performed at 4 ℃.

Analysis of the mice thymus and spleen indices

The thymus and spleen indices were calculated in accordance with the following formula and in accordance with the method described previously [33, 34]:

Thymus or spleen index = (weight of thymus or spleen/body weight) × 100%

Statistical Analysis

When compared with the radiation the control data were expressed as the mean ± S.D. The statistical evaluation was performed using the student's *t-test.*

RESULTS

Preparation SNAAP and Gas Chromatography -Mass Spectrometry Analysis (GC–MS)

This outcome signified that the SNAAP was successfully sulfated by IR. A characteristic of the chromatogram, depicted in figure 1, showed that the polysaccharide was a typical heteropolysaccharide and was composed of ribose, rhamnose, arabinose, xylose, mannose, glucose, and galactose, with a respective molar ratio at: 0.1; 0.1; 0.1; 0.4; 0.7; 1.0; 0.2. It was evident that the predominant composition monosaccharide, within the polysaccharide, was neutral mannose and glucose residues.

Radioprotections of SNAAP on White Blood Cell Count in human peripheral blood

X-ray-irradiated significantly decreased the WBC count in human peripheral blood (Group II) compared with control group($p<0.01$). Human peripheral blood pretreated with the SNAAP (20, 60 and 100 µg/mL), progressively increased the WBC count in human peripheral blood when compared with X-ray-irradiated Peripheral blood (Group II)($p<0.01$). Likewise, the SNAAP treatment increased the white blood cell count in the human peripheral blood samples compared with Groups I. However, a significant increase in the WBC count occurred by the addition of the 60 µg/mL SNAAP to the peripheral blood sample (Group IV).

Effect on the morphology of peripheral white blood cells

This research also found that the SNAAP pretreated with 60 µg/mL increased the WBC more than that of in Group III and Group V compared with Group II. Also observed was the SNAAP (60 µg/mL) effect on changes in the different type of white blood cells (figure 2). This outcome further indicated that the SNAAP also alleviated different white blood cell damage against radiation (Group II).

The result of the SNAAP (60 µg/mL, Group IV) on the effect of morphology of peripheral white blood cells as followed:

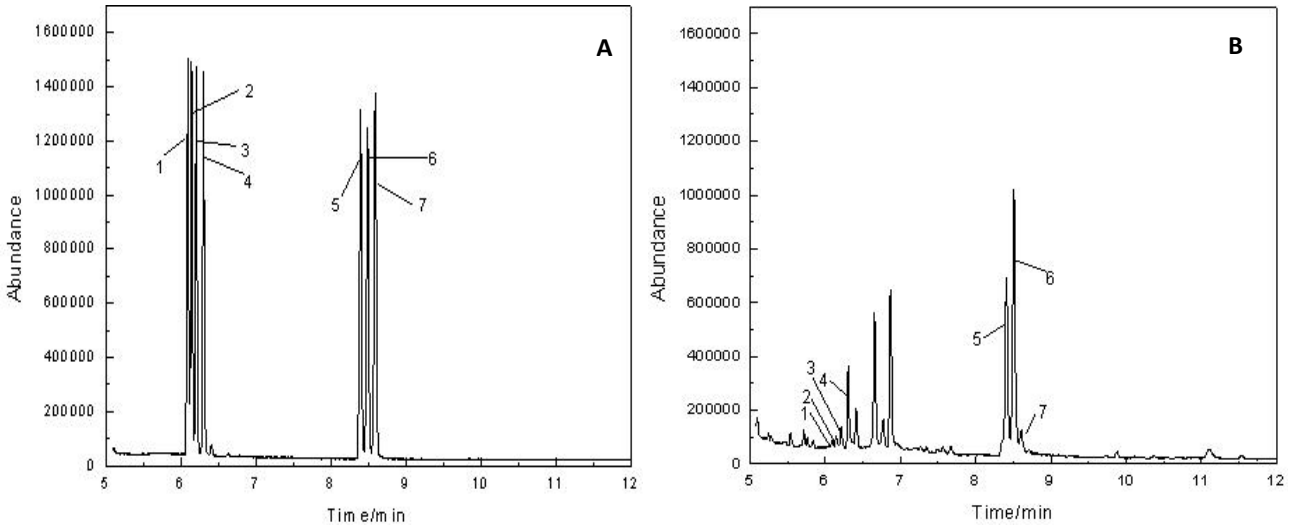

Figure 1. GCMS Chromatography: A) GC-MS analysis of STANDARD, B) GC-MS analysis of SNAAP.

The effect of the SNAAP (60 μg/mL) on the morphology of peripheral white blood cells in the peripheral blood samples is shown in figure 3. Figure 4 shows the different morphology of the human peripheral white blood cells. Blood film was prepared from the whole human blood 24 hours and 48 hours post-radiation by observing the white blood count morphology. Of note, when compared with Group II 24 hours post-radiation, there occurred a marked decrease in the amount of degeneration in the white blood cell count 48 hours post-radiation. Moreover, the SNAAP treatment increased the count of Pelger-Huet anomaly in the peripheral blood samples 48 hours post-radiation compared with Groups II 24 hours post-radiation. As well, the SNAAP treatment increased the Pelger-Huet count anomaly. That is to say, the Pelger-Huet with toxic granulation, the band neutrophil with vacuoles, and the abnormal monocyte in the peripheral blood samples 48 hours post-radiation compared with Group II 24 hours post-radiation. However, the administration of the SNAAP to the peripheral blood samples significantly increased the atypical lymphocytes count.

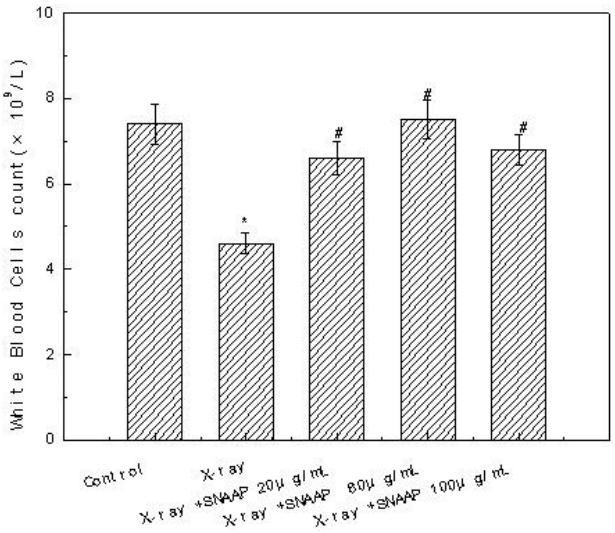

Figure 2. White blood cell count among different groups (n=3)
* vs.control p<0.01, **vs.control p<0.05, # vs.model p<0.01, ## vs. model p<0.05

Effects of SNAAP on γ-ray radiation-induced Spleen Lymphocytes viability

As shown in figure 5, the effects of the SNAAP on the Wistar rats' spleen lymphocyte proliferation were examined 24 hours after radiation. Radiation significantly decreased the proliferation of the spleen lymphocytes as determined by the MTT assay compared control group($p<0.01$). After pretreatment with the SNAAP (20, 60and 100 μg/mL) for 12 hours prior to radiation, the cell viability was improved in a concentration dependent manner compared with control group($p<0.01$, $p<0.05$). While after pretreatment with the SNAAP (20 and 60 μg/mL) for 12 hours prior to radiation, the cell viability was improved in a concentration dependent manner compared with the irradiated group($p<0.05$). The cell viability of the irradiated control group was

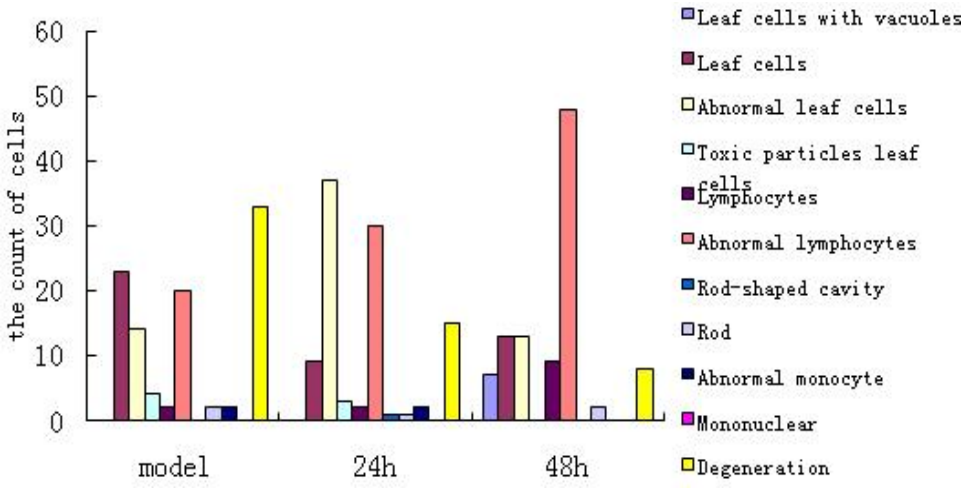

Figure 3. SNAAP(60 μg/mL) on morphology of peripheral white blood cells.

approximately 53.04 ± 7.29%. However, the cell viability of the SNAAP pretreatment groups, with SNAAP at a concentration of 20, 60, and 100 μg/mL, was respectively: 79.84 ± 9.30%, 83.21 ± 10.60% and 46.93 ± 6.85%. The study further demonstrates the treatment of the spleen lymphocytes cells, with radiation, could lead to cell death via apoptotic processes, whereas the SNAAP prevented radiation-induced lymphocytes cells apoptosis in a dose-dependent manner.

γ-ray radioprotection of SNAAP against radiation-induced injury of mice

Effect of SNAAP on mice' body weight and biochemistry measurement

One day after receiving 6 Gy of radiation, this treatment resulted in a decreased body weight in the mice when compared to the non-irradiated control group (table 1). The irradiated mice lost weight after receiving radiation and began to gain weight less than the control mice, at low, middle, and a high dose of the polysaccharides treated mice. The control mice continued to gain weight and reached 30.51 ± 2.93g a day after receiving radiation while the irradiated mice reached their weight of 28.23 ± 3.94g.

There were differences between the body weights of both groups. Significant increased body weight in the three polysaccharides groups was observed in comparison with radiation group a day after 6 Gy of radiation. This result indicates that the oral administration of a dose-dependent SNAAP protected against the radiation-induced decreased body weight.

As shown in table 1, it was found that the spleen and thymus indexes in the irradiated control group (Group II) were lower than those found in the non-irradiated control group.

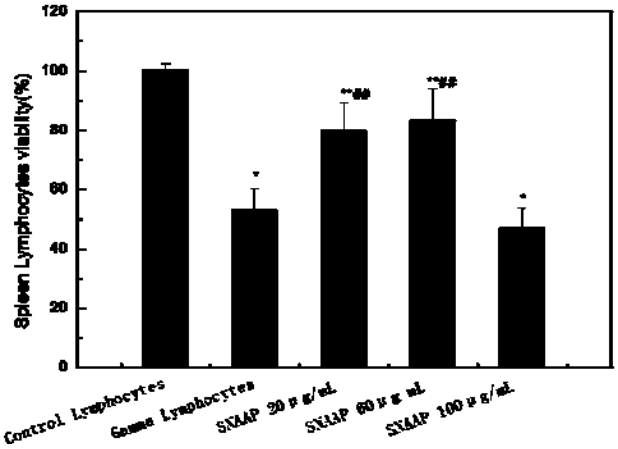

Figure 5. Effects of SNAAP on γ-ray radiation-induced Spleen Lymphocytes viability. Spleen Lymphocytes were treated with SNAAP at 20-100 μg/mL, followed by γ-ray irradiation at 8 Gy 12 hours later. Cell viability was assessed 24 h after RT. Cell proliferation in RT groups is expressed as a percentage of the proliferation of un-irradiated control cells. Values are shown as mean±S.D. n=6); * vs.control p<0.01, **vs.control p<0.05, # vs.model p<0.01, ## vs. model p<0.05

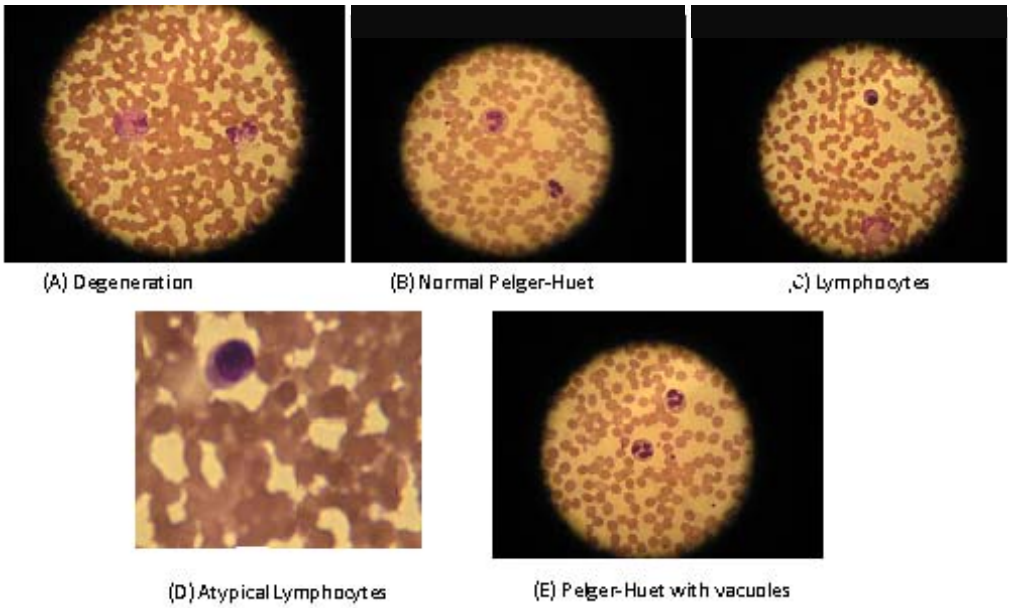

Figure 4. morphology of several kinds of peripheral white blood cells.

Independent treatment with SNAAP at low, middle, and at a high dose increased the spleen and thymus indexes in the irradiated mice (Groups III, IV, and V) compared to the irradiated control mice (Group II) ($p<0.01$, $p<0.05$). The SNAAP treatment, at doses of 50, 100 and 200 mg/kg/d, implies that SNAAP effectively stimulated the immune system of the irradiated mice.

MDA level, GSH-Px, and MPO activity in mice blood one day after receiving 6 Gy of radiation

Table 2 illustrates that one day after receiving 6 Gy of radiation the mice who received radiation treatment showed an increased MDA level, a decreased GSH-Px, and an MPO activity level in their blood. Furthermore, the MDA level, the GSH-Px activity and the MPO activity in the irradiated control of the mice's blood was 5.89 ± 0.24 nmol/mL, 176.62 ± 10.39 U/mL and 127.24 ± 1.60 U/L. When compared against MDA level, the GSH-Px activity and the MPO activity of the non-irradiated control group, the variance was 5.64 ± 0.91 nmol/mL, 189.61 ± 2.60 U/mL and 118.24 ± 3.52 U/mL. Table 2 also illustrates the significant statistical difference between the non-irradiated control group and the irradiated control group. Table 2 also shows that radiation-induced injury has a close relationship with the alteration of the free radical in biology and that polysaccharides treatment demonstrably protected the experiment mice from radiation-induced injury in a dose-dependent manner. Moreover, when ompared with the irradiated control group, the MDA level, the GSH-Px activity and the MPO activity in whole blood of the three groups of the polysaccharides treated mice significantly decreased or increased in comparison with the mice's irradiated control group ($p<0.05$) (table 2). This outcome validates that SNAAP decreased radiation-induced injury by its strong free radical scavenging activity.

Table 1. Effects of SNAAP administration on body weight (B.W.) and the immune organ indexes of mice a day after 6 Gy radiation.

GROUP	Dose (mg/kg/d)	Initial B.W.(g)	Final B.W.(g)	Spleen index (%)	Thymus index (%)
I	0	23.73±0.25	30.51±2.93	0.428±0.085	0.180±0.079
II	6 Gy	24.50±0.34	28.23±3.94	0.158±0.013[c]	0.063±0.031[c]
III	50+6 Gy	24.65±1.24	31.09±2.34	0.214±0.031[b]	0.133±0.054[a]
IV	100+6 Gy	24.24±0.10	30.22±4.21	0.169±0.020[a]	0.141±0.010
V	200+6 Gy	24.27±1.91	29.79±3.22	0.169±0.018[a]	0.084±0.035

Body weight was recorded at 1-day following 6 Gy radiation to the whole body (10 mice for each group). Results are mean ± SD of 10 parallel measurements and followed by the Student's t-test. I, Non-irradiated control; II, Irradiated control(6 Gy); III, Irradiated +polysaccharides(50mg +6 Gy); IV, Irradiated +polysaccharides(100mg +6 Gy); V, Irradiated +polysaccharides(200mg +6 Gy).
[a] $p<0.05$ compared with irradiated control (II, 6 Gy). [b] $p<0.01$ compared with irradiated control (II, 6 Gy).
[c] $p<0.05$ compared with Non-irradiation control (I). [d] $p<0.01$ compared with Non-irradiation control (I).

Table 2. MDA level, GSH-Px, and MPO activity in the mice blood a day after 6 Gy of radiation.

GROUP	Dose(mg/kg/d)	MDA (nmol/mL)	GSH-Px (U/mL)	MPO(U/L)
I	0	5.64±0.91	189.61±2.60	118.24±3.52
II	6 Gy	5.89±0.24	176.62±10.39	127.24±1.60[c]
III	50+6 Gy	5.26±0.87[ad]	175.32±9.09	123.56±6.48
IV	100+6 Gy	5.61±0.45	177.92±11.69	122.48±6.36
V	200+6 Gy	5.88±1.04[c]	181.82±21.17	126.92±1.45[a]

Results are means ± SD of 10 parallel measurements and followed by the Student's t-test. I, Non-irradiated control; II, Irradiated control(6 Gy); III, Irradiated +polysaccharides(50mg +6 Gy); IV, Irradiated +polysaccharides(100mg +6 Gy); V, Irradiated +polysaccharides(200mg +6 Gy).
[a] $p<0.05$ compared with irradiated control (II, 6 Gy). [b] $p<0.01$ compared with irradiated control (II, 6 Gy).
[c] $p<0.05$ compared with Non-irradiation control (I). [d] $p<0.01$ compared with Non-irradiation control (I).

Effects of SNAAP on mice radiation-reduced WBC, RBC, and PLT

As illustrated in table 3, one day following radiation the white-cell count was (1.21 ± 0.31) 10^9/mL and in turn was significantly lower than in the non-irradiated control group (5.15 ± 0.64)109/mL ($p<0.01$). This outcome shows that the radiation-induced injury had seriously weakened the mice and their intrinsic immunomodulatory function and generated inflammation. As well, the polysaccharides oral administration markedly protected the mice from the radiation-induced injury in a dose-dependent manner. Following the first day, after being irradiated the white cell counts were (1.64 ± 0.43) 10^9/mL, (1.52 ± 0.50) 10^9/mL, and (2.07 ± 0.35) 10^9/mL in the low, middle, and high dose of the polysaccharides-treated mice were higher than those found in the irradiated control group (table 3). Although, the counts failed to return to normal at the low, middle and a high dose of the SNAAP can both increase RBC and PLT counts.

DISCUSSION

The exogenous sources of free radicals come from environmental contaminants such as from pollution and cigarette smoking, ionizing and ultra violet radiation. Wholebody exposure of mice to gamma radiation leads to diminution of tissue antioxidant defense systems; increases the peroxidative damage to membrane lipids and damages the haematopoietic systems[35]. An antioxidant is any material that do something as free radical scavenger that prevents smash up of cells caused by corrosion[36]. When an antioxidant becomes a free radical itself, it is not harmful because it is able to provide somewhere to stay the change in electrons without appropriate reactive.

There has been a major progress in the area of radio-protective agents since the early 1980s in terms of biological and mechanistic evaluation of the large number of natural antioxidants or synthesized compounds. Plants with radio protective properties have been shown almost invariably to possess antioxidant bimolecular. The radio-protective effect of antioxidant molecules such as polysaccharides from Acanthopanax senticosus and the ethanolic extract from Pileamicrophylla (L.) and, as well, from Lycium barbarum, Ganoderma lucidum, and dehydrozingerone and their incumbent polysaccharides; have largely attributed to the anti-oxidative properties of these compounds [37-39]. Many synthetic compounds have also been found to protect biological systems against radiation induced damage, for example Tempol (TPL) prevented the radiation induced depletion in RBC and total WBC counts, glutathione content in blood and bone marrow cellularity. TPL also protected the tissue antioxidant system and membrane lipids from the radiation-induced damages. An enhanced spleen colony formation and spleen weight recovery were also observed in radiation exposed mice administered with TPL[35]. Cimetidine was found to be more protective against mortality induced by radiation in mice compared to famotidine by Mozdarani (2008). This effect of cimetidine might be due to its

Table 3. Effects of SNAAP on radiation-reduced white cells count, red cell and platelet count.

Group	Dose(mg/kg/d)	RBC($\times10^{12}$/L)	WBC($\times10^9$/L)	PLT ($\times10^9$/L)
I	0	9.29±0.82	5.15±0.64	603±74
II	6 Gy	8.61±1.05	1.21±0.31[d]	502±100
III	50+6 Gy	9.67±0.88	1.64±0.43	575±93
IV	100+6 Gy	9.94±0.65[a]	1.52±0.50	619±74
V	200+6 Gy	9.59±0.52	2.07±0.35[b]	596±108

Results are means ± SD of 10 parallel measurements and followed by the Student's t-test. I, Non-irradiated control; II, Irradiated control(6 Gy); III, Irradiated +polysaccharides(50mg+6Gy); IV, Irradiated +polysaccharides(100mg +6 Gy); V, Irradiated +polysaccharides(200mg +6 Gy).
[a] $p<0.05$ compared with irradiated control (II, 6 Gy). [b] $p<0.01$ compared with irradiated control (I, 6 Gy).
[c] $p<0.05$ compared with Non-irradiation control (I). [d] $p<0.01$ compared with Non-irradiation control (I).

immunomodulatory role and thus protecting bone marrow and lymphoid tissue injuries following whole body gamma irradiation[40]. There is increasing interest in naturally occurring antioxidants for use in foods and nutra-ceutiacl products to replace synthetic antioxidants[41]. It has been suggested that natural antioxidants are safer and more healthful than synthetic antioxidants used in foods [42,43].

Protective effects of SNAAP prepared by the laboratory [22] on hematologic injury induced by radiation including three models:

1. An evaluation of X-ray radioprotection of SNAAP in human peripheral blood model,
2. γ-ray radioprotection of SNAAP in cultured rat spleen lymphocytes; and, in addition, γ-ray radioprotection of SNAAP against radiation-induced injury to the laboratory mice, that is, from *in vitro* to in *vivo*.

The administration of the SNAAP to the peripheral blood samples significantly increased the atypical lymphocytes count and revealed that the SNAAP also alleviated different white blood cell damage caused by radiation. A combination of treatments of the SNAAP and X-ray resulted in no significant effect or variation in the manual white blood count in the human peripheral blood. Nevertheless, the use of radiation-responsive targets is recommended for dose-assessment applications.

The pleasing therapeutic effect of polysaccharides on many diseases may also be fully explained by the mechanism of enhancing immunity. This study, (rat spleen lymphocytes) further demonstrates the treatment of the spleen lymphocytes cells, with radiation, could lead to cell death via apoptotic processes, whereas the SNAAP prevented radiation-induced lymphocytes cells apoptosis in a dose-dependent manner. Moreover, that radiation-induced injury has a close relationship with the alteration of the free radical in biology and that polysaccharides treatment demonstrably protected the experiment mice from radiation-induced injury in a dose-dependent manner.

This outcome validates that SNAAP decreased radiation-induced injury by its strong free radical scavenging activity. The results of this research also demonstrate that the SNAAP possesses beneficial radiation protection properties that partly attribute to the SNAAP having robust anti-infection activity.

Tsiapali *et al.* found that phosphated and sulfated glucan showed greater antioxidant ability. This finding indicated the polyelectrolytes, such as glucan sulfated, or phosphate, may increase scavenging activity[44]. One mechanism of protective effect of SNAAP is by reducing the potency of ROS, particularly $O_2^{-\cdot}$. Just what Dekkers mentioned that there are three categories of naturally occurring antioxidant: SOD, CAT and GSH-Px [45]. SNAAP can increase antioxidant enzymes activity (GSH-Px) (p>0.05) in the blood compared with model group. Moreover, SNAAP can significantly decrease MDA level (p<0.05) compared with model group. Cellular damage is closely related with lipid peroxidation and causes aging, carcinogenesis and many other diseases and glutathione peroxides removes H_2O_2 and lipid peroxides, which indicated the level of oxidative stress state inside cells [46]. The second mechanism of protective effect of SNAAP is that SNAAP can increase antioxidant enzymes activity and decrease MDA level. Finally, SNAAP effectively stimulated the immune system of all radiated mice.

CONCLUSION

The study's results demonstrate that it is possible to synthesize from an *Auricularia auricular* derivative and a sulfate of neutral polysaccharides with an average DS of the SNAAP to attain 0.78. The study's results also show that the SNAAP also alleviates different white blood cell damage against radiation. The research confirms the effects of the SNAAP on the spleen lymphocytes proliferation. The implications from the study's findings suggest the need for further study to clarify the subset population numbers of lymphocyte pretreatment with the SNAAP. Nevertheless, regardless of this outcome there is a need for further *in-vitro* and clinical studies to clarify the

WBC count action mechanisms and to improve the morphology and the peripheral of the white blood cell interaction by increasing cell viability by a pretreatment with the SNAAP. The oral administration of a dose-dependent SNAAP protected against radiation-induced decreased body weight and effectively stimulated the immune system of the irradiated mice. This outcome likewise demonstrates that the SNAAP decreased radiation-induced injury partly by its strong free radical scavenging activity.

To conclude, this research validates that the sulfation of neutral polysaccharides extracted from *Auricularia auricular* (SNAAP) mushrooms, have significant therapeutic potential, and represent a rich source for future scientific discovery and the development of novel compounds of medical value.

ACKNOWLEDGMENTS

This research was supported by a grant from the Chinese National High Technology Research and Development Program (863 Program, Grant No. 2007AA100404). The author expresses her thanks to Professor Robert J. Tuck from Australia who edited and refined the paper.

Author Disclosure Statement

No competing financial interests exist.

REFERENCES

1. Adhvaryu MR, Srivastav SP, Vaniawala SN, Reddy MN (2008) A comparative study of radioprotection by four Indian medicinal herbs against genotoxicity induced by sub-lethal gamma irradiation in Swiss albino mice. *Iran J Radiat Res, **6**: 19-30.*
2. Mikkelsen RB and Wardman P (2003) Biological chemistry of reactive oxygen, nitrogen, and radiation-induced signal transduction mechanisms. *Oncogene, **22**: 5734-5754.*
3. Lee JH, Kim SY, Kil IS, Park JW (2007) Regulation of ionizin gradiation-induced apoptosis by mitochondrial NADP+-dependent isocitrate dehydrogenase. *J Biol Chem, **282**:13385-13394.*
4. Zhang R, Kan Kyoung Ah g, Piao MJ, Ko Dong Ok, Wang ZH, Lee In Kyung, *et al.* (2008) Eckol protects V79-4 lung fibroblast cells against-ray radiation-induced apoptosis via the scavenging of reactive oxygen species and inhibiting of the c-Jun NH_2-terminal kinase pathway. *Eur J Pharmacol, **59**: 114-123.*
5. Petushkova NA, Bachmanova GI, Archakov AI *(*1996) 7-Alkoxyphenoxazone and 7-Alkoxycoumarin O-dealkylation by human peripheral blood lymphocytes. *In vitro toxicolo **9**: 291-295.*
6. Baron JM, Zwadlo-Klarwasser G, Jugert F, Hamann W, Rübben A, Mukhtar H, Merk HF (1998) Cytochrome p.450 1B1: A major p.450 isoenzyme in human blood monocytes and macrophage subsets. *Biochem Pharmacol,* **56**: 1105-1110.
7. Dey A, Parmar D, Dhawan A, Dash D, Seth P KA (2002) Cytochrome p450 2 E1 dependent catalytic activity and lipid peroxidation in rat blood lymphocytes. *Life Sci, **71**: 2509-2519.*
8. Sokolov AA (1961) The effect of ionising radiation on stored blood. *Problems of Hematology and Blood Transfusion, **6**: 186-189.*
9. Williams JP, Brown SL, Georges GE, Hauer-Jensen M, Hill RP, Huser AK, Kirsch DG (2010) Animal Models for Medical Countermeasures to Radiation Exposure. *Radiat Res*, ***173**:557-578.*
10. Hosseinimehr SJ (2007) Trends in the development of radio-protective agents. *Drug Discov Toda, **12**: 794-805.*
11. Maurya DK, Devasagayam, TPA, Nair, CK (2006) Some novel approaches for radioprotection and the beneficial effect of natural products. *Indian J Exp Biol, **44**:93-114.*
12. Prasad NR, Mahesh T, Menon VP, Jeevanram RK, Pugalendi K V (2005) Photoprotective effect of sesamol on UVB-radiation induced oxidative stress in human blood lymphocytes *in-vitro*. *Environ Toxicol Phar, **20**: 1-5.*
13. Prasad NR, Ramachandran S, Pugalendi KV, Menon VP (2007) Ferulic acid inhibits UV-B-induced oxidative stress in human lymphocytes. *Nutr Res, **27**: 559-564.*
14. Yamini K and Gopal V, (2010) Natural Radio-protective Agents against Ionizing Radiation–An overview. *Int J Pharmtech Res, **2**: 1421-1426.*
15. Ooi VEC and Liu F (2000) Immunomodulation and anticancer activity of polysaccharide protein complexes. *Curr Med Chem, **7**: 715-729.*
16. Li X L and Zhou GA (2007) Preparation of polysaccharides from Acanthopanax senticosus and its inhibition against irradiation-induced injury of rat. *Carbohyd Polym, **67**: 219 -226.*
17. Yan PS, Luo XC, Zhou Q (2004) RAPD molecular differentiation of the cultivated strains of the jelly mushrooms, Auricularia auricula and A. polytricha. *World J Microb Biot, **20**: 795-799.*
18. Chang JS, Kim HJ, Bae JT, Park SH, Kim SE, Kim OM, Lee KR (1998) Inhibition effects of A.auricula judae-judae methanol extract on lipid peroxidation and liver damage in benzo(α)pyrene-treated mice. Korean society of food science and nutrition, ***27**:712-717.*
19. Mau, J L, Chao GR, Wu KT (2001) Antioxidant properties of methanolic extracts from several ear mushrooms. *J Agr Food Chem, **49**: 5461-5467.*
20. Luo YC, Chen G, Li B, Ji BP, Guo Y, Tian F (2009) Evaluation of anti-oxidative and hypolipidemic properties of a novel

functional diet formulation of Auricularia auricula and Hawthorn. *Innovative Food Science and Emerging Technologies,* ***10****: 215-221.*

21. Wu Q, Tan Z, Liu H, Gao L, Wu S, Luo J, *et al.* (2010) Chemical characterization of Auricularia auricula polysaccharides and its pharmacological effect on heart anti-oxidant enzyme activities and left ventricle ejection fraction in aged mice. *Int J of Biol Macromol,* ***46****: 284-288.*
22. Zhang H, Wang ZY, Yang L, Yang X, Wang X, Zhang Z (2011) *In-vitro* Antioxidant Activities of Sulfated Derivatives of Polysaccharides Extracted from Auricularia auricula. Int J Mol Sci, ***84****: 3287-3202.*
23. El-Missiry MA, Fayed TA, El-Sawy MR, El-Sayed AA (2007) Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. *Ecotoxicol Environ Saf,* ***66****: 278-286.*
24. Honda S, Suzuki S, Kakehi K, Honda A, Takai T (1981) Analysis of the monosaccharide compositions of total non-dialyzable urinary gly-coconjugates by the dithioacetal method. J Chromatogr, ***226****: 341-350.*
25. Oades JM (1967) Gas-liquid chromatography of alditol acetates and it application to the analysis of sugars in complex hydrolysates. J. Chromatogr, ***28****: 246-252.*
26. Johnes TM and Albersheim P (1972) A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharide. Plant Physiol, ***49****: 926-936.*
27. Mozdarani H, Taheri A, Haeri SA (2007) Assessment of radiop-rotective effects of amifostine on human lymphocytes irradiated in vitro by gamma-rays using cytokinesis-blocked micronucleus assay. *J Radiat Res*, ***5:*** *9 -16.*
28. Becton-Dickinson (1998) *Unopette WBC determination for manual methods*; Rutherford, N.J.: Becton, Dickinson, and Company.
29. Berend H (2002) Blood film preparation and staining procedures. *Clins Lab Med,* ***22****: 1-14.*
30. Bain B J (2007) Blood Sampling and Blood Film Preparation and Examination, in Blood Cell A Practical Guide, Fourth Edition, Barbara J. Bain, Oxford, UK.
31. Habu S (1986) in Zaku Seikagaku Jikkenkoza (The Japanese Biochemical Society, ed.) ***5****: 169.*
32. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res,* ***47****: 936-941.*
33. Ma M, Liu GH, Yu ZH, Chen G, Zhang X (2009) Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in-vivo. *Food Chem,* ***113****: 872-877.*
34. Zalys R, Zagon IS, Bonneau RH, Lang CM, McLaughlin PJ (2000) *In-vivo* effects of chronic treatment with [Met5]-enkephalin on hematological values and natural killer cell activity in athymic mice. *Life Science,* **66**: 829-834.
35. Ramachandran L, Nair CKK (2012) Radioprotection by tempol: Studies on tissue antioxidant levels, hematopoietic and gastrointestinal systems, in mice whole body exposed to sub- lethal doses of gamma radiation. Iran J *Radiat Res*, ***10****: 1-10.*
36. Youngston R (1994) The Antioxidant Health Plan. UK: Harper Collins Publisher.
37. Li X L, Zhou AG, Li XM (2007) Inhibition of Lycium barbarum polysaccharides and Ganoderma lucidum poly-saccharides against oxidative injury induced by c-irradiation in rat liver mitochondria. *Carbohyd Polym,* ***69****: 172-178.*
38. Parihar VK, Dhawan J, Kumar S, Manjula SN, Subramanian G, Unnikrishnan MK, et al. (2007) Free radical scavenging and radio-protective activity of dehydrozingerone against whole body gamma irradiation in Swiss albino mice. *Chem Biol Interact,* ***170****:49-58.*
39. Prabhakar KR, Veerapur VP, Bansal P, Parihar VK, Reddy Kandadi M, Bhagath Kumar P, *et al.* (2007) Antioxidant and radio-protective effect of the active fraction of Pilea microphylla (L.)ethanolic extract. *Chem Biol Interact,* ***165****: 22-32.*
40. Mozdarani H, Salimi M, Froughizadeh M (2008) Effect of cimetidine and famotidine on survival of lethally gamma irradiated mice. Iran *J Radiat Res,* ***5****:187-194.*
41. Lizcano LJ, Bakkali F, Ruiz-Larrea MB, Ruiz-Sanz JI (2010) Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. *Food Chem,* ***119****:1566-1570.*
42. Reddy CVK, Sreeramulu D, Raghunath M (2010) Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Res Int*, ***43:****285-288.*
43. Blomhoff R (2005) Dietary antioxidant and cardiovascular disease. *Curr Opin in Lipidol*, ***16****: 47-54.*
44. Tsiapali E, Whaley S, Kalbfleisch J, Ensley HE, Browder IW, Williams DL (2001) Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. *Free Radical Bio Med,* ***30:****393-402.*
45. Dekkers JC, Doornen LJP, Kemper HCG (1996) The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. *Sport Med,* ***21:*** *213-238.*
46. Yagi K (1987) Lipid peroxides and human disease. *Chem Phys Lipids,* ***45:****337-341.*