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Preparation of polysaccharides from *Acanthopanax senticosus* and its inhibition against irradiation-induced injury of rat

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Abstract

Acanthopanax senticosus grows in many provinces in china and has long been used as a traditional medicine in china. This paper reports on the extraction of polysaccharides from *A. senticosus* and its effect of irradiation-protection. 15 Gy X-rays irradiation was delivered to rats. Polysaccharides from *A. senticosus* were administered before and after irradiation to examine its inhibition against irradiation-induced injury. Results indicate that in comparison with non-irradiated controls, irradiation of 15 Gy resulted in a significant reduction of rats' body weight (BW), food and water intake, white cell counts (WC), activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and increase of MDA level 10–20 days after being irradiated although after that these indexes start to slowly return to normal ($p < 0.05$, $p < 0.01$). Oral administration of polysaccharides from *A. senticosus* dose-dependently reduced the irradiation-induced injury on rats studied, showed a protective effect against irradiation-induced loss of BW, WC, food and water intake, reduce MDA level and raise antioxidase activity (SOD, GSH-Px) ($p < 0.05$, $p < 0.01$). It may be concluded that polysaccharides from *A. senticosus* possess good irradiation-protective effect.

Keywords: Polysaccharides from *Acanthopanax senticosus*; Extraction; Irradiation injury; Irradiation-protective effect; GSH-Px; Superoxide dismutase

1. Introduction

Plants have been utilized since time immemorial for curing diseases. Even today, nearly 70% of the world's population is dependent on plants for handling their health related problems (Fabricant & Farnsworth, 2001). With the recognition that normal tissue protection in radiotherapy is as important as the destruction of the cancer cells, the focus of protection research became more therapy oriented. Since the pioneering work of Patt, Tyree, Straube, and Smith (1949), that cysteine protected mice and rats against radiation-induced sickness and mortality, several chemical compounds and their analogues have been screened for their radioprotective ability. However, the practical applicability of the majority of these synthetic compounds remained limited, owing to their high toxicity at their optimum protective doses (Sweeney, 1979). A number of plants have been utilized successfully for the treatment of free radical-mediated diseases in humans such as rheumatoid arthritis, atherosclerosis, cancer, Alzheimer's disease, Parkinson's disease, aging and several other conditions including inflammatory diseases (Singh et al., 2001). It is, therefore, reasonable to expect that plants may contain certain compounds that can protect against radiation-induced reactive oxygen species (ROS)-mediated damage. The herbal drugs offer an alternative to the synthetic compounds that have been considered either nontoxic or less toxic than their synthetic counterparts. This has given impetus to screen herbs for their radioprotective ability. The compound formulations are extensively used in the Ayurvedic system of medicine to counteract the toxicity of one herb with the other. The herbal preparation Liv. 52, which has been widely used to treat liver disorders, has

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been reported to protect mice against radiation-induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in the peripheral blood cell counts, prenatal development and radiation-induced chromosome damage (Jagetia & Ganapathi, 1991; Saini, Kumar, Jagetia, & Saini, 1984a, 1984b; Saini, Kumar, Uma, & Saini, 1985; Saini & Saini, 1985). Certain other herbal preparations like brahmarasayana, narasimharasayana, ashwagandharasayana, and amrithaprasham, a group of herbal preparations used to improve the general health, have also been reported to reduce the radiation-induced lipid peroxidation in the liver, and leucopenia in mice (Kumar, Kuttan, & Kuttan, 1996). Abana, a composite herbal preparation, clinically used in India as a cardioprotective agent has also been reported to protect the mice bone marrow against the radiation-induced micronuclei formation (Jagetia & Ganapathi, 1991). The extracts of certain plants like *Ocimum sanctum Panax ginseng* And *Chlorella vulgaris* have been reported to protect mice against the radiation-induced mortality (Singh, Ashu, Tiku, & Kesavan, 1995; Zhang, Sigdestad, Gemmell, & Grdina, 1987).

Acanthopanax senticosus (AS) is a typical Chinese herb, its roots are an important Chinese folk medicine for the treatment of a variety of human diseases, such as isochemic heart diseases, hypertension, rheumatism, allergies and diabetes and tumor, etc. There is growing interest in the structures of these biomedical active molecules in this plant. An aqueous extract of the root bark has been shown to protect mice from both stress-induced physiological and physical changes (Nishiyama, Cho, Kitagawa, & Saito, 1994). Further investigations have demonstrated that the AS extract or its components such as chlorogenic acid (CHA) and syringaresinol di-*o*-D-glucoside (SYG) markedly prevent the occurrence of gastric ulcer in rats exposed to restraint stress in water (RSW) for 7 h (Fujikawa, Yamaguchi, Morita, Takeda, & Nishibe, 1996). Saponins have been reported to be responsible for the biomedical activities (Shao, Kasai, Xu, & Tanaka, 1988, 1989). Davydov and Krikorian (2000) reported that the herb included various compounds such as acanthosides, eleutherosides, senticoside, triterpenic saponin, flavon, vitamins and minerals, and they are related to its diverse biological activities. Yi et al. (2001) found that *A. senticosus* root may possess effective anti-anaphylactic activity. Yoon et al. (2004) reported good antitumor and immunomodulatory activities of an aqueous extract (GF100) of *A. senticosus*. Fujikawa et al. (2005) reported that an aqueous extract of the stem bark of *A. senticosus* (AS) from Japan prolonged the swimming time of rats in a forced swimming test (Nishibe et al., 1990). Han et al. (2003) isolated a new immunostimulatory polysaccharide from a cell culture of *A. senticosus*. Polysaccharide fractions from *A. senticosus* were previously shown to increase lymphocyte proliferation, T-dependent antibody responses and macrophage phagocytosis (Shen et al., 1991). Furthermore, this polysaccharide significantly inhibited the growth of Sarcoma 180 and prolonged the survival time of tumor-bearing mice.

In recent decades, polysaccharides isolated from botanical sources (mushrooms, algae, lichens and higher plants) have also attracted a great deal of attention in the biomedical arena because of their broad spectrum of therapeutic properties and relatively low toxicity (Paulsen, 2001; Tzianabos, 2000; Wasser, 2002). While our understanding of the mechanism of action of these substances is still developing, it appears that research of polysaccharides is facing a promising future (Tzianabos, 2000). Most polysaccharides derived from higher plants are relatively non-toxic and do not cause significant side effects, which is a major problem associated with immunomodulatory bacterial polysaccharides and synthetic compounds. Thus, plant polysaccharides are ideal candidates for therapeutics with immunomodulatory, anti-tumor, wound-healing action and irradiation-protective effect (Lazareva et al., 2002; Ovodov, 1998). It has not been reported yet whether body weight, water and food intake of animals receiving irradiation can be affected by administration of polysaccharides isolated from *A. senticosus*, although Study on irradiation-protection of the polysaccharides have been conducted (Liao, Chen, & Yang, 2005). In the present study, we analyzed radiation-induced rats' body weight, food and water intake alteration, oxidation damage and examined the effect of the polysaccharide from *A. senticosus* (ASP) on irradiation-induced injury.

2. Materials and methods

2.1. Chemicals

Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween 20), α-tocopherol, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^-$), 3-(2-pyridyl)-5,6-bis-(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), and trichloracetic acid (TCA) were purchased from Sigma Chemical Co. The reagents and kits for detecting enzyme activation were purchased from Nanjing Jiancheng Bioengineering Institute (NJBI, Nanjing, China). All others unlabelled chemicals and reagents were analytical grade.

2.2. Animals

Male Wistar rats, weighing 236–294 g, were purchased from the institute of TS microorganism. They were kept in cages under an alternating 12-h light/dark cycle, and maintained with free access to food and water.

2.3. Preparation of polysaccharides from A. senticosus

Acanthopanax senticosus (root), collected in a hillside 30 km away from our institute, was used as the raw material. The roots was air-dried, milled, homogenized in a single lot and stored under dry conditions before use. The roots were milled in a Manesco and Ranieri knife mill to pass through a 0.5 mm screen. Approximately 100 g of

milled sample was extracted with 2000 ml distilled water for 6 h on a shaking incubator at 80 °C and 140 rpm. Protein impurity in polysaccharides may be removed by the sevag method (Arif & Gupta, 2003). Briefly, same volume of mixture solution of chloroform and *n*-butanol (4/1) was added into the extraction solution, shaken up, stood for overnight. Supernatant and denatured protein were removed. The residual material (polysaccharides) was cooled, filtered through a porous glass filter number 3 and concentrated under vacuum down to 70 ml in a rotative evaporator of 100 rpm. Ninety-five percent ethanol of same volume was then added into the concentrated solution, centrifugated (4000*g*) to precipitate polysaccharides, and the procedure was duplicated for five times. The precipitated polysaccharides extraction solution of all five times were pooled and was washed three times with a 1:1 mixture of diethyl ether and acetone and then lyophilized to obtain polysaccharides from *A. senticosus*. Total sugars were determined by a phenol–sulfuric acid method, using D-glucose as standard (Chaplin, 1986).

2.4. Irradiation

Irradiation was conducted by the method of Nagler, Barak, and Nagler (2000) with some modification. Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital, 30 mg/kg, weighed. The animals were whole-bodily irradiated with a single 15 Gy dose delivered by a 250 kV therapeutic X-ray tube (Philips Medical System Inc.) operated at 235 kV and 15 mA, with an output of 1.26 Gy/min at 54 cm and a copper filter. All irradiation was carried out between 10:00 a.m. and 2:00 noon. Control animals were anaesthetized, but had no radiation delivered.

2.5. Polysaccharides treatment

Polysaccharides from *A. senticosus* of varying dose (200, 400, 600 mg/kg) dissolved in sterile saline was orally given 50 min before and after irradiation on the basis of our past experience. Control animals were orally given same volume of sterile saline alone.

2.6. Effect of polysaccharides from A. senticosus on irradiation-induced change of rats' body weight, food and water intake

In this experiment, the rats were housed separately in individual cages and freely access to food and water. During 2 months after receiving irradiation, body weight and food and water intake were measured at 10 days intervals for each rat. Water intake was assessed by measuring the volume of water consumed. White blood-cell counts were taken on the 10th day after receiving irradiation.

At 60 days after receiving irradiation, rats' eyeball were removed for blood sampling. Serum MDA level, GSH-Px activity in whole blood and SOD activity in red cells of irradiated rats were measured.

2.7. Biochemistry measurement

The magnitude of oxidation in liposomes after various incubation periods was quantified by measurement of oxidation index (Terao, Shibata, & Matsushita, 1988). Oxidation of lipids involves the peroxidation of their polyunsaturated fatty acid resulting byproducts such as conjugated diene, which shows absorbance at 233 nm. Oxidation index is the ratio of absorbance at 233 and 215 nm corresponding with the concentration of diene conjugate and phospholipid, respectively. Radiation-induced peroxidation in liposomes was measured spectrophotometrically by thiobarbituric acid (TBA) reaction following standard protocol with a slight modification (Pandey & Mishra, 2004). Malondialdehyde (MDA), one of major degradation products of lipid peroxidation, can better reflect the degree of lipid peroxidation. For determination of the level of MDA in non-radiated control and radiated rats' serum, 0.3 ml serum samples were mixed with 1 ml TBA reagent (0.5% TBA dissolved in 10% trichloro acetic acid in distilled water) followed by heating at 80–85 °C for 20 min. Samples cooled at ice temperature were centrifuged at 12,000 rpm for 10 min and absorbance of supernatant was determined at 532 nm using a 8453 UV–Visible Spectrophotometer (purchased from Tian Jing Analysis instrument Co. Ltd). At the same time, same volume of physiological saline was measured by identical way as blank control. Result was expressed as nmol/ml.

Determination of SOD activity was by the method of Ginnopolitis and Ries (1977). The reaction mixture was comprised 3.3 mL, 50 mmol/L sodium phosphate buffer (pH 7.5), 9.9 mmol/L methionine, 57 mol/L nitroblue tetrazolium (NBT) and the appropriate volume of blood sample, and the reaction was initiated by light. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease in the SOD-inhibitable NBT reduction. SOD activity was expressed as U/ml.

Measurement of GSH-Px activity was based on the following principle: GSH-Px catalyzes the oxidation of glutathione by cumene hydroperoxide (Schepetkin, Faulkner, Nelson-Overton, Wiley, & Quinn, 2005). In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. The enzyme unit of GSH-Px (U) is defined as the number of micromoles of reduced NADPH oxidized per minute at 37 °C by 1 ml of blood sample under standard assay conditions. Measurements were performed by an automatic analyzer (MEK-6102 Hematology Analyzer, purchased from Nihon company) according to the Randox application procedure. GSH-Px activity in whole blood was expressed as U/ml.

2.8. Statistical analysis

The significance of the results was evaluated by *t*-test for differences in means ($p < 0.05$ level of significance). Values are presented as means ± SD.

3. Result and discussion

3.1. Ultraviolet and infrared spectrum characteristics of polysaccharides from A. senticosus

The polysaccharides extract was not be detected significant absorption peak in the wavelength of 280 nm and 260 nm by a ultraviolet spectrophotometer, which indicates that protein and nucleic acid impurity have been reduced to the lowest amount. Typical polysaccharides absorption peaks at 3372, 2931, 1611, 1419 and 1062 cm^{-1} in infrared spectrum were observed, which confirms higher purity of polysaccharides extract.

3.2. Determination of maximum absorption peak

Maximum absorption peak were determined by method of Habibi, Mahrouz, and Vignon (2005) with some slight modification. Two milliliters of standard glucose dilution solution, 5.0 ml sulfuric acid, and 1.0 ml, 6% phenol were mixed with constant stirring in a glass vessel of 100 ml, and then stood for 20 min at room temperature. At last, absorbance values were recorded by 722 spectrophotometer at the wavelength range of 380–600 nm. At the same time, 2.0 ml distilled water was measured as blank control according to identical way. Scan spectrogram curve may be drawn by absorbance values and wavelength (Fig. 1). From Fig. 1, we could observe the maximum absorptance peak at 590 nm. Therefore, we chose 590 nm as the optimal measure wavelength to raise measure precision.

3.3. Drawing of the standard curve of glucose

A volume of 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 ml glucose solution prepared were, respectively, taken and in turn added into 20 cuvettes of 5 ml cubage, and then supplied water up to 2.0 ml. Then 1.0 ml, 6% phenol and 5.0 ml sulfuric acid solution were in turn added into the cuvettes, shaken up and stood for 20 min at room temperature. At last, absorbance values were recorded by 722 spectrophotometer at the wavelength of 490 nm. Two milliliters of reagent blank was used as blank control according to identical way. Standard curve of glucose may be drawn. Abscissa stand for glucose concentration (μg/ml); vertical coordinates stand for OD value.

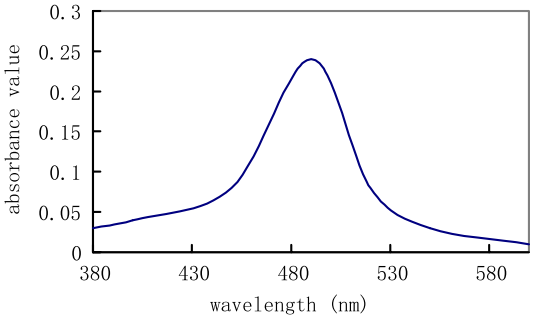

Fig. 1. Scan spectrogram curve for polysaccharides from *Acanthopanax senticosus*.

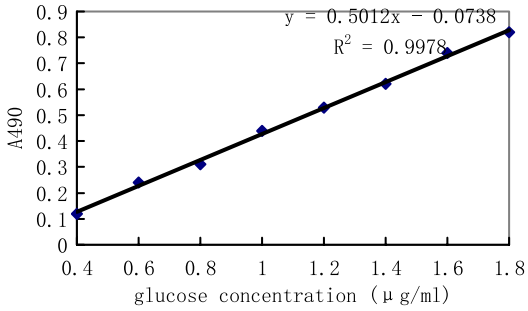

Fig. 2. Standard curve of glucose solution.

Standard curve of glucose was shown in Fig. 2. Regression equation: $Y = 0.5012X - 0.0738$; $R^2 = 0.9978$.

Results indicate that there existing a good linearity between glucose concentration and absorbance within the concentration range of 0.4–1.8 μg/ml.

3.4. Determination of conversion factor

Twenty milligram polysaccharides from *A. senticosus* was dissolved in a capability flask of 100 ml, shaken up and supplied to scale with distilled water. Two milliliters of the solution prepared was accurately taken and measured by identical way to 3.2. Conversion factor may be calculated by the following equation:

$$f = \frac{W_1}{C \times D}$$

where W_1 represents weight of polysaccharides from *A. senticosus*; C represents amount of glucose in polysaccharides solution; D represents dilution multiple of polysaccharides solution. Result shows that f is 4.5128.

3.5. Determination of polysaccharides content

Fifty milligram of polysaccharides from *A. senticosus* was accurately taken and together with proper amount of distilled water, added into a capacity flask of 250 ml with constant stirring to dissolve, supplied to the scale with distilled water. One milliliter of the result solution was accurately taken, added into a glass tube of 5 ml, and supplied with distilled water to 2.0 ml. Then absorbance of polysaccharides conducted by identical way to 3.2. At the same time, reagent blank was measured by identical way as blank control. Total sugar content in polysaccharides extract may be calculated by the following equation:

$$\text{Total sugar content}(\%) = \frac{C \times D}{W_2} \times f \times 100\%$$

where C is glucose amount (μg) in sample (polysaccharides extract); D is dilution multiple of sample. f is conversion factor. W_2 is weight of sample.

Result (total sugar content) by calculating is 89.75% which is in line with result of ultraviolet and infrared spectrum analysis.

3.6. Effect of polysaccharides from A. senticosus on body weight and intake of food and water after rats' receiving irradiation

It could be found that irradiation of 15 Gy resulted in a significantly decreased body weight in comparison with the non-irradiated control group two months after receiving irradiation (Table 1). The irradiated rats, which had lost weight immediately after receiving irradiation, began to gain weight after the 10th day, while the non-irradiated control rats had gained weight throughout the 2-month study period (Table 1). The control rats continued to gain weight and reached 520.17 ± 18.37 g at 2 months, while the irradiated rats reached their maximal weight 421.08 ± 18.93 g at 2 months. There are significant difference between body weight of both groups ($p < 0.05$; $p < 0.01$). Significant increased body weight in three groups of polysaccharides could be observed in comparison with irradiation group in two months ($p < 0.05$; $p < 0.01$). Result indicates that oral administration of polysaccharides from *A. senticosus* dose-dependently protected against the irradiation-induced decreased body weight. Water and food intake were significantly lower after receiving irradiation, reaching its lowest value 69.11 ± 10.03 ml and 41.57 ± 3.59 g at 20th day in the irradiated control rats compared with 113.54 ± 13.95 ml and 75.04 ± 8.49 g in the non-irradiated control rats ($p < 0.01$) (Tables 2 and 3). After that, the values started to rise, but still was lower than water and food intake of non-irradiated control rats at same date ($p < 0.05$, $p < 0.01$). Polysaccharides treatment significantly improved water and food intake of irradiated rats at a dose-dependent manner. Significantly increased water and food intake in three groups of polysaccharides treatment rats could be observed in comparison with irradiated control rats. At the 60th day, The values basically increase to normal (Tables 2, 3).

3.7. Effect of polysaccharides from A. senticosus on serum MDA level, GSH-Px activity in blood and SOD activity in red cells of rats' receiving irradiation

Plants with radioprotective properties have been shown almost invariably to possess antioxidant biomolecules. The radioprotective effect of antioxidant molecules such as eugenol from *Zingiber officinalis*, genistein from *Glycine*

Table 1
Effect of polysaccharides from *Acanthopanax senticosus* on rats' body weight (g/rat) for 2 months after 15 Gy irradiation

Group	Days						
	0	10	20	30	40	50	60
1	266.54 ± 9.18	337.39 ± 10.18	391.40 ± 28.79	440.14 ± 11.58	466.13 ± 20.78	500.48 ± 23.24	520.17 ± 18.37
2	263.37 ± 19.22	227.05 ± 17.49a	313.01 ± 22.18b	345.07 ± 22.74b	387.66 ± 31.87b	401.34 ± 26.71b	421.08 ± 18.93b
3	267.66 ± 21.58	238.93 ± 11.24	338.13 ± 20.14c	379.16 ± 23.84c	403.77 ± 27.06c	410.31 ± 31.67	463.17 ± 20.01c
4	268.74 ± 11.44	254.91 ± 16.91c	358.77 ± 28.12d	389.43 ± 29.42d	428.27 ± 28.55d	445.68 ± 33.15d	489.48 ± 24.14d
5	264.33 ± 25.26	257.93 ± 23.64c	361.36 ± 25.38d	397.65 ± 30.63d	433.43 ± 33.46d	453.11 ± 29.75d	492.71 ± 44.37d

Body weight was recorded at 10-day interval for 2 months following 15 Gy irradiation to the whole body (10 rats for each group). Results are mean ± SD of 10 parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $p < 0.05$ when compared to control. 1, non-irradiation control; 2, irradiation control (15 Gy); 3, polysaccharides + irradiation (200 mg + 15 Gy); 4, polysaccharides + irradiation (400 mg + 15 Gy); 5, polysaccharides + irradiation (600 mg + 15 Gy). [a]$p < 0.05$, [b]$p < 0.01$, when compared to non-irradiation control; [c]$p < 0.05$, [d]$p < 0.01$, when compared to irradiation control.

Table 2
Effect of polysaccharides from *Acanthopanax senticosus* on irradiation-induced rats' water intake (ml/day rat)

Group	Days						
	0	10	20	30	40	50	60
1	103.49 ± 10.83	113.90 ± 11.73	113.54 ± 13.95	121.65 ± 15.93	126.44 ± 19.37	121.54 ± 12.37	126.53 ± 14.31
2	104.97 ± 12.83	81.34 ± 7.57b	69.11 ± 10.03b	93.76 ± 6.11b	107.35 ± 11.97a	116.44 ± 9.95	119.68 ± 16.30
3	119.36 ± 16.77	87.54 ± 11.09	92.49 ± 15.38c	110.71 ± 11.19c	117.44 ± 9.11	119.41 ± 13.37	127.58 ± 14.47
4	119.48 ± 12.71	91.36 ± 7.07	101.31 ± 9.12d	115.08 ± 7.57c	120.22 ± 9.13c	126.73 ± 14.80	130.63 ± 15.37
5	114.36 ± 8.24	92.94 ± 6.48c	111.37 ± 10.01d	119.35 ± 12.05c	123.76 ± 11.86c	130.11 ± 9.62c	128.61 ± 10.53

Water intake was recorded daily for 2 months following 15 Gy irradiation to the whole body (10 rats for each group). Total water intake of each 10 days was divided by 10 to obtain water intake/day rat. Results are means ± SD of 10 parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $p < 0.05$ when compared to control. 1, non-irradiation control; 2, irradiation control (15 Gy); 3, polysaccharides + irradiation (200 mg + 15 Gy); 4, polysaccharides + irradiation (400 mg + 15 Gy); 5, polysaccharides + irradiation (600 mg + 15 Gy). [a]$p < 0.05$, [b]$p < 0.01$, when compared to non-irradiation control; [c]$p < 0.05$, [d]$p < 0.01$, when compared to irradiation control.

Table 3
Effect of polysaccharides from *Acanthopanax senticosus* on irradiation-induced rats' food intake (g/day rat)

Group	Days						
	0	10	20	30	40	50	60
1	79.34 ± 8.11	69.13 ± 7.14	75.04 ± 8.49	82.02 ± 6.17	79.71 ± 8.14	93.44 ± 9.70	91.43 ± 10.07
2	81.07 ± 3.45	44.17 ± 5.08b	41.57 ± 3.59b	46.01 ± 7.15b	61.66 ± 6.03a	81.03 ± 8.37	83.37 ± 8.01
3	74.71 ± 6.74	48.73 ± 8.91	43.57 ± 5.83	61.19 ± 7.13c	73.41 ± 8.93c	86.44 ± 6.30	90.04 ± 7.23
4	77.44 ± 9.05	50.63 ± 9.21	47.76 ± 9.15	78.68 ± 9.76d	96.49 ± 7.35d	94.37 ± 7.84	92.63 ± 9.38
5	80.68 ± 8.37	52.41 ± 5.42	50.74 ± 5.98	80.12 ± 8.03d	93.35 ± 7.65d	95.66 ± 9.05	93.55 ± 8.11

Food intake was recorded daily for 2 months following 15 Gy irradiation to the whole body (10 rats for each group). Total food intake of each 10 days was divided by 10 to obtain food intake/day rat. Results are means ± SD of 10 parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $p < 0.05$ when compared to control. 1, non-irradiation control; 2, irradiation control (15 Gy); 3, polysaccharides + irradiation (200 mg + 15 Gy); 4, polysaccharides + irradiation (400 mg + 15 Gy); 5, polysaccharides + irradiation (600 mg + 15 Gy). [a]$p < 0.05$, [b]$p < 0.01$, when compared to non-irradiation control; [c]$p < 0.05$, [d]$p < 0.01$, when compared to irradiation control.

max; orientin, vicenin and ursolic acid from *Ocimum sanctum*, curcumin from *Curcuma longa*, bixin from *Bixa orelleana*, quercetin from *Podophyllum hexandrum*, β-carotene from the heattolerant algae *Dunaliella baradwiil*, luteolin from *Aspalanthus linearis*, allicin from *Allium sativum*, glycyrrhizin from *Glycyrrhiza glabra*, caffeine from *Coffea arabica*, flavan-3-ols (procyanidins) from *Vitis vinifera*, flavone glycosides and terpenes from *Ginkgo biloba*, silymarin from *Silybum marianum*, epigallocatechin from 'Thea viridis' (green tea percolate) and melatonin (*N*-acetyl-5-methoxytryptamine) from *Hypericum perforatum, Silybum marianum, Lycium* spp. has largely been attributed to the antioxidative properties of these compounds (Abraham, Sarma, & Kesavan, 1993; Alaoui-Youssefi, Lamproglou, Drieu, & Emerit, 1999; George, Hebbar, Kale, & Kesavan, 1999; Inano & Onoda, 2002; Landauer, Srinivasan, & Seed, 2003; Reiter & Tan, 2002). In the present study, the obtained data showed that polysaccharides from *A. senticosus* possessed strong antioxidation activity. As be shown in Table 4, increased serum MDA level, decreased GSH-Px activity in whole blood and SOD activity in red cells of rats' receiving irradiation were observed 2 months after receiving 15 Gy irradiation. Serum MDA level, GSH-Px activity in whole blood and SOD activity in red cells in irradiated control rats were 11.33 ± 1.02 nmol/ml, 24.22 ± 5.27 U/ml and 70.11 ± 7.82 U/ml, respectively, in comparison with 6.20 ± 1.47 nmol/ml, 31.23 ± 2.91 U/ml and 96.47 ± 5.56 U/ml in the non-irradiated controls. There is significant statistical difference between non-irradiation control and irradiation control ($p < 0.01$) (Table 4). This indicates that irradiation-induced injury keeps a close relationship with alteration with free radical in biology. Polysaccharides treatment obviously protected experiment rats from irradiation-induced injury at a dose-dependent manner. Compared with the irradiated control, serum MDA level, GSH-Px activity in whole blood and SOD activity in red cells of three groups of polysaccharides treatment rats were significantly decreased or increased in comparison with the irradiated control rats ($p < 0.05$, $p < 0.01$) (Table 4). This demonstrates that polysaccharides from *A. senticosus* decrease irradiation-induced injury partly by its strong free radical scavenging activity.

3.8. Effect of polysaccharides from *A. senticosus* on irradiation-reduced white cells count

At present, study of polysaccharides mainly focus on theirs immunomodulatory function. For example, polysaccharides derived from 35 plant species among 22 different families have been shown to enhance macrophage function. In particular, these compounds have been shown to increase macrophage cytotoxic activity against tumor cells and microorganisms, activate phagocytic activity, increase reactive oxygen species (ROS) and nitric oxide (NO) production, and enhance secretion of cytokines and chemokines, such as tumor necrosis factor (TNF-α), interleukin (IL)-1h, IL-6, IL-8, IL-12, IFN-g and IFN-h2 (Fujikawa et al., 2005; Jain, Singh, Mishra, & Vyas, 2005). In fact, wonderful therapeutic effect of polysaccharides on many diseases may also be fully explained by the mechanism of enhancing immunity.

Table 4
Serum MDA level, GSH-Px activity in whole blood and SOD activity in red cells 2 months after 15 Gy irradiation to the whole body

Group	Dose	SOD (U/ml)	MDA (nmol/ml)	GSH-Px (U/ml)
Non-irradiation control	0	96.47 ± 5.56	6.20 ± 1.47	31.23 ± 2.91
Irradiation control	15 Gy	70.11 ± 7.82b	11.33 ± 1.02b	24.22 ± 5.27b
Irradiation + polysaccharides	200 mg + 15 Gy	83.75 ± 9.43c	9.27 ± 1.57c	30.45 ± 4.46c
Irradiation + polysaccharides	400 mg + 15 Gy	89.66 ± 10.07c	8.58 ± 1.77c	33.81 ± 4.13d
Irradiation + polysaccharides	600 mg + 15 Gy	97.22 ± 7.02c	7.05 ± 1.28d	40.66 ± 5.78d

Results are means ± SD of 10 parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $p < 0.05$ when compared to control. [b]$p < 0.01$, when compared to non-irradiation control; [c]$p < 0.05$, [d]$p < 0.01$, when compared to irradiation control. 10 rats for each group.

Table 5
Effect of polysaccharides from *Acanthopanax senticosus* on white cells count following irradiation

Group	Dose	White cells count (10^6/ml)
Non-irradiation control	0	16.64 ± 1.71
Irradiation control	15 Gy	3.24 ± 0.94b
Irradiation + polysaccharides	200 mg + 15 Gy	7.17 ± 1.97d
Irradiation + polysaccharides	400 mg + 15 Gy	8.44 ± 1.37d
Irradiation + polysaccharides	600 mg + 15 Gy	16.23 ± 1.54d

Results are means ± SD of 10 parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $p < 0.05$ when compared to control. [b]$p < 0.01$, when compared to non-irradiation control; [d]$p < 0.01$, when compared to irradiation control. Ten rats for each group.

As illustrated in Table 5, the white-cell count 10 days following irradiation was (3.24 ± 0.94) 10^6/ml, significantly lower than in the non-irradiated controls (16.64 ± 1.71) 10^6/ml ($p < 0.01$). This indicates that irradiation-induced injury has seriously weaken rats' intrinsic immunomodulatory function and generated inflammation. Oral administration with polysaccharides markedly protected the rats from the irradiation-induced injury at a dose-dependent manner. At the 10th day after being irradiated, the white-cell counts were (7.17 ± 1.97) 10^6/ml, (8.44 ± 1.37) 10^6/ml and (16.23 ± 1.54) 10^6/ml, respectively, in low, middle and high dose of polysaccharides-treated animals, which were significantly higher than those of irradiated controls ($p < 0.05$) (Table 5). The counts returned to normal at high dose of polysaccharides treatment rats at the 10th day after being irradiated. Results of our work show that polysaccharides from *A. senticosus* possess good irradiation protection property, which partly attributes to their strong anti-infection activity.

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