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Radioprotective effects of active compounds of *Acanthopanax senticosus* from the Lesser Khingan Mountain range in China

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Abstract: Bioactive compounds including polysaccharides, flavones, syringin and eleutheroside E were extracted from wild *Acanthopanax senticosus* to get the purities of 88.4%±3.2%, 90.8%±2.0%, 92.5%±1.5% and 82.7% ±4.7% respectively. *In vitro* antioxidant activities and *in vivo* anti-radiation activities of the compounds were investigated and compared. Results demonstrated that polysaccharides and flavones extracted from *A. senticosus* were more effective than syringin and eleutheroside E in their radical scavenging activity *in vitro*. *In vivo* studies showed that polysaccharides and flavones were also effective in protecting mice from heavy ion radiation induced tissue oxidative damage. Furthermore, the activities of polysaccharides and flavones in repressing expression changes of radiation response proteins including heat shock protein, disulfide-isomerase and glutathione S-transferase, were also identified by our results. These radioprotective effects were more significant when polysaccharides and flavones were administered together.

1. Introduction

Radiation exposure can cause many kinds of disease by increasing oxidative pressure in living organisms. Among all the possible sources of radiation, heavy ion radiation is the most harmful in terms of risks to humans, as it is usually more than 10-fold more effective in causing cell damage than other sources of radiation such as X-rays, γ-rays, or electrons [1,2]. In recent decades, chemicals and functional compounds derived from natural products have been the focus of investigation with the aim of identifying new radioprotective medicines and foods that may improve human health [3,4]. Amongst these natural products, some traditional Chinese medicines such as herbal drugs have been used for millennia. Nowadays, more and more herbal drugs are being investigated to identify the

functional compounds within them and to determine their biological activities [5]. The potential of these herbal drugs to be exploited as new functional foods, such as anti-cancer and anti-radiation foods, is becoming the subject of increased attention.

Acanthopanax senticosus (AS) is one of the most well known traditional Chinese herbal drugs which is constantly spread and widely cultivated in North Asia. AS has been used for the treatment of many different kinds of disease and disease symptoms, such as hypertension [6], hepatitis [7], inflammation [8] and ischemic disease [9]. In recent years it has been revealed that the main active compounds in AS are eleutheroside, flavones and polysaccharides. The biological activity of these compounds has been widely investigated in mammals, plants and insects [10]. AS extract has been shown to enhance gut health in weaning piglets [11], exert protective effects on gut immunity of *Drosophila* [12], and to reduce oxidative stress [13]. These findings suggest that there is potential for AS and its active compounds to be explored as health care products with anti-radiation activities. However, to date, little research attention has been given to this subject.

The Lesser Khingan Mountain range is one of the three largest forested areas in China and is located in the transitional zone between the boreal and temperate forests. More than half of the wild AS used by the Chinese comes from the Lesser Khingan range. *Acanthopanax senticosus* originating from the Lesser Khingan range (LAS) has been most widely used because it grows in the natural environment. In the present study, we separated the active compounds of polysaccharides, flavones, syringin and eleutheroside E from LAS. The activities of *in vitro* anti-oxidative and *in vivo* anti-radiation properties of these compounds were investigated and compared.

2. Experimental Section

2.1. Chemicals

Syringin, eleutheroside E, D-glucose and rutin were purchased from National lnstitutes for Food and Drug Control (Beijing, China). α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from SigmaeAldrich (Buchs, Switzerland). Ferrous chloride, polyoxyethylene sorbitan monolaurate (Tween 20), a-tocopherol, 1,1- dipheny l- 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. The reagents and kits for detecting enzyme activation were purchased from Nanjing Jiancheng Bioengineering Institute of China. All others unlabelled chemicals and reagents were analytical grade.

2.2. Plant material

Acanthopanax senticosus (LAS) was collected in Lesser Khingan Mountain, Heilongjiang, China, in September 2010. The AS was identified by Prof. Yu Jiabin (Herb Identification and Medicinal Plant Teaching and Research Section, Heilongjiang University of Chinese Medicine). The 4-year old LAS were chosen randomly. The root, stem, leave and fruit of LAS was separated and stored at -80℃ after freeze-dried.

2.3. Preparation of active compounds from Lesser Khingan Mountain range derived Acanthopanax senticosus (LAS)

2.3.1. Extraction of polysaccharides

A total of 200g of each of the following: roots, stems, leaves and fruits of LAS were individually milled and extracted with distilled water at 65℃ for 3h. After being centrifuged, the supernatants were concentrated by evaporation. The solution was then precipitated again with three volumes of 95% ethanol and placed at 4℃ overnight [14,15]. The polysaccharide fragment was obtained by filtration of the solution with a 0.45μm membrane and washed three times with 95% ethanol, followed by acetone, and then dried at room temperature.

2.3.2. Preparation of flavone

A total of 300g of each of the following: roots, stems, leaves and fruits of LAS were each extracted three times with a total of 3000 ml 70% ethanol for 2h per time. The extraction was filtered and concentrated in a rotary evacuator to obtain the ethanol extract of LAS (EELAS) [16]. Flavones were separated and purified from the EELAS by passing through an AB-8 macroporous adsorption resin column [17]. The sample was then eluted with ethanol. The ethanol phase was evaporated under vacuum and then oven dried at 60℃ to isolate the extracts.

2.3.3. Separation of syringin and eleutheroside E from the EELAS

For further isolation of syringin and eleutheroside E, the EELAS were subjected to column chromatograph on silica gel (Qingdao Haiyang Chemical Plant, China) and eluted with a step gradient chloroform-methanol solvent system (50:1, 40:1, 20:1, 10:1, 5:1, 2:1, 1:1 and 1:2). Component 1 (from 5:1 elution) and component 2 (from 1:2 elution) were collected to obtain syringin and eleutheroside E respectively after reducing pressure to recover the solvent. The compounds were then dried in vaccum at 40℃.

2.3.4. Calculation of the yield of active compounds

The extraction yield was calculated, in each case, as the weight of the dried compound as a percentage of the dried plant tissue using the equation: dried sample%=(g_{sample}/g_{tissue})×100, where g_{sample} is the weight of the dried compound (polysaccharides, flavones, syringing, or eleutheroside E), and g_{tissue} is the weight of the plant tissue (root, stem, leaves or fruit) in dried weight.

2.3.5. Identification and quantification of polysaccharides and flavonoids (flavones)

Total polysaccharide content was determined by the phenol-sulfuric acid method, using D-glucose as the standard [18]. Briefly, the polysaccharide powder extracts were dissolved in distilled water to obtain a 1mg/mL solution, then phenol and concentrated sulfuric acid were added sequentially. After vortexing and then standing for 30 min at room temperature, the absorbance of the solution was measured by spectrophotometer (Shimadzu-UV-2450, Shanghai, China) at 490 nm. Polysaccharide content was expressed as grams of D-glucose equivalents per 100g of dried LAS (%).

Dried flavonoid extracts were smashed and sieved using a 20 mesh sieve. A total of 0.5g of the resulting fine powder was then weighed and dissolved in ethanol. After circulation reflux, filtration and vacuum concentration, the residue was dissolved in methanol and diluted to 100 ml. 0.1 mL of the

residue solution was measured accurately and dissolved in methanol to 20 ml. The concentration of total flavones was then detected at 257nm by UV spectrophotometer (Shimadzu-UV-2450, Shanghai, China) [19]. Rutin solutions were used for constructing the calibration curve ($y = 30.69x + 0.0274$; $R^2 = 0.9992$). Total flavonoid content of the extract was expressed as grams of rutin equivalents per 100g of dried LAS (%).

2.3.6. Determination of syringin and eleutheroside E

The content of syringin and eleutheroside E in the samples was detected by HPLC. An Agilent 1100 series liquid chromatograph (Agilent Technologies, USA) equipped with a quaternary gradient pump and a UV detector was used. A HPLC method was developed using a reversed-phase C18 column (Agilent-TC, 250mm×4.6mm, 5μm i.d.) with the column temperature at 30℃. Sample injection quantity was 20μL; the elution solvent consisted of water (A, with 0.1% phosphoric acid) and acetonitrile (B) with the following gradient program: 0-10min, 90%A; 10-20min, 85%A; 20-30min, 80%A; 30-40min, 75-90%A; 40-50min, 90%A. The flow rate was kept at 1 ml/min, and the absorbance was measured at a wavelength of 220nm for Syringin, 207nm for eleutheroside E [20,21]. The retention times are 12min and 15min for Syringin and eleutheroside E respectively. The content was expressed in each case as grams of syringin or eleutheroside per 100g of dried LAS (%).

2.4. Measurement of in vitro antioxidant capacity

2.4.1. Scavenging activity of DPPH radical

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to a modified method by Brand-Williams *et al.* [22]. In each case, the extracted samples of the active compounds were dissolved in distilled water to form the sample solution in final concentrations of 0.1, 0.5, 1, 2, 3, 4, 5 and 10mg/ml respectively. Equal volumes of the sample solutions and 250μM DPPH ethanol solution were then mixed. After incubation at 37℃ for 30min, the absorbance of the mixture was measured at 517nm. The percentage inhibition was calculated against a control sample.

2.4.2. Scavenging activity of ABTS radical

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity of the extracted samples was estimated using the procedure described by Sanchez Gonzalez *et al.* [23] with some modifications. The ABTS radical solution was produced by creating an ABTS aqueous solution (ABTS 7mmol/L and potassium persulphate 2.45mmol/L), and the mixture was incubated in the dark at room temperature for 16h. After incubation, the ABTS radical solution was diluted with PBS (pH 7.0) to an absorbance of 0.70 (±0.02) at 734nm. The extracted samples were then dissolved in distilled water to form sample solutions in final concentrations of 0.1, 1, 2, 3, 4, and 5 mg/ml, respectively. The sample solutions were then added to the ABTS radical solution in a ratio of 1:20, and the resulting mixture was incubated for 6min at room temperature. The absorbance was measured at 730 nm. The scavenging activity on ABTS radical was calculated against a control sample.

2.5. Animal experiments

A total of 72 male mice of 6-8 weeks old, weighing 20.0±5g, were purchased from the Health and Anti-epidemic Service, Lanzhou, China, and maintained under conditions of controlled temperature (23±2℃) and humidity (50±5%), and a 12h light/dark cycle. The mice were allowed at least 1 week to adapt to the environment before being used for experiments. Mice were randomly divided into 12 groups (n=6 per group). These groups comprised of: normal control group, irradiated control group, polysaccharides (5mg/day) treatment group, polysaccharides (10mg/day) treatment group, flavones (5mg/day) treatment group, flavones (10mg/day) treatment group, eleutheroside E (5mg/day) treatment group, eleutheroside E (10mg/day) treatment group, syringin (5mg/day) treatment group, syringin (10mg/day) treatment group, polysaccharides (1.5mg/day) plus flavones (3.5mg/day) treatment group, and polysaccharides (3mg) plus flavones (7mg) treatment group.

The active compound samples to be tested were dissolved in normal saline (NS). Mice were then exposed daily to aqueous solutions of the active compounds at the above described dosing levels over a two week period. The mice in the control groups were given NS daily on the basis of equal volume. All mice, except those from the normal control group, were irradiated by $^{12}C^{6+}$ with the mean linear energy transfer (LET) value of 62.2 KeV/μm at a dose of 2Gy and a dose rate of 0.1Gy/min. Each mouse was placed in a separate plastic container (20cm×20cm×100cm), and exposed to head irradiation. Mice of each group were euthanized by cervical dislocation at three days after irradiation. The procedures of the experiment were strictly performed according to the generally accepted international rules and regulations.

2.6. MDA content and SOD activity

Malondialdehyde (MDA) content of mice tissue homogenates was assessed spectrophotometrically using the thiobarbituric acid test [24]. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured spectrophotometrically based on inhibition of the photochemical reduction of nitroblue tetrazolium [25,26].

2.7. Two-dimensional gel electrophoresis (2-DE) analysis

2.7.1. 2-DE of liver homogenates

Mouse livers were homogenized and sonicated. After centrifugation, the supernatant was assayed for protein content [27]. Samples containing equal quantities of 1000μg of protein were diluted with rehydration buffer to obtain (in each case) 340μL of solution and then incubated overnight with nonlinear Immobiline Dry Strips (18cm; pH 3-10 non-linear). The samples were then separated in the 1st dimension by isoelectric focusing (IEF). The immobilized pH gradient (IPG) strips were then incubated with equilibration buffer for 15min followed by incubation in the same buffer with the dithiothreitol (DTT) replaced by 2.5% w/v iodoacetamide for 15min. The strips were applied to the surface of 12% w/v SDS-PAGE gels for image analysis [28,29].

2.7.2. Image analysis of 2-DE gels

The gels were stained with colloidal Coomassie Blue and scanned using a GS710 calibrated imaging densitometer (BioRad, Hemel Hempstead, UK). TIFF format images were analysed using ImageMaster TM 2D Elite software, version 4.01 (Amersham Pharmacia Biotech, Buckinghamshire, England).

2.7.3. Identification of proteins from 2DE gels

Protein spots of interest were excised from Colloidal Coomassie Blue-stained 2DE gels and were subjected to tryptic digestion. Peptide mass fingerprints were averaged and searched using the Group Based Prediction System database (GPS) (Applied Biosystems, Life Technologies, USA) with MASCOT (Matrix Science, London, UK). The searching parameters were as follows: database: NCBInr; species: mice; retrieval mode: combined; enzyme: trypsin; mass error range: PMF 100ppm, MS/MS 0.8KU. Trypsin self-degradation and contaminant peaks were removed manually [30].

3. Results and Discussion

3.1. Content detection of active compounds from LAS

Water soluble polysaccharides were extracted from the root, stem, leaves and fruits of LAS. The extraction yield of the polysaccharides across the different LAS plant tissues was 7.5%-17.1%. The total carbohydrate content in the extracted polysaccharides was determined by the phenol-sulfuric acid method. The yield of ethanol extract across the different LAS plant tissues was 33.5%-45.3%. The yields of flavones, syringin and eleutheroside E from the ethanol extracts of the different LAS plant tissues were determined by spectrophotometric method or HPLC. The content of each active compound in each of the different LAS tissue types is listed in Table 1. In each case, the yield was calculated according to the dry biomass of root, stem, leaves and fruits of LAS respectively. As shown in Table 1, the contents of the active compounds in the root and stem are generally higher than those in the leaves and fruit.

Table 1. Content of the active compounds in different tissues of LAS

Compounds	Content of the active compounds (%)			
	Tissue of Lesser Khingan Mountain *Acanthopanax senticosus*			
	Root	Stem	Leaf	Fruit
Polysaccharides	6.05±0.25	8.09±0.37	4.12±0.19	3.08±0.13
Flavones	14.25±0.66	9.26±0.45	4.02±0.17	9.58±0.50
Syringin	1.44±0.06	1.86±0.08	0.09±0.01	0.02±0.001
Eleutheroside E	3.17±0.12	4.14±0.25	0.18±0.01	0.04±0.002

3.2. Isolation and purification of active compounds from LAS

The purity of the polysaccharides extracted from the different tissues of LAS was calculated to be 88.4%±3.2%. Flavones, syringin and eleutheroside E were isolated from EELAS by the method of column chromatography. Flavones were separated by macroporous adsorption resin. The purity was calculated to be 90.8%±2.0%, detected by the spectrophotometric method. Syringin and eleutheroside E were separated and purified by silica gel column chromatography. The content of each was detected by HPLC using syringin and eleutheroside E as the respective standards. The purity of the extracted syringin and eleutheroside E was 92.5%±1.5% and 82.7% ±4.7% respectively.

3.3. In vitro antioxidant activities of active compounds from LAS

Recently, a wide range of spectrophotometric assays have been adopted to measure the antioxidant capacity of active compounds from drugs and foods. The most popular methods used have been the ABTS assay and DPPH assay [31,32,33]. In the present study, extracted active compounds including syringin, eleutheroside E, flavones and polysaccharides were diluted to appropriate concentrations to evaluate their anti-oxidative activity in terms of their DPPH and ABTS radical scavenging activity.

In the case of the DPPH assay, as expected, the active compounds exhibited radical scavenging activities in a concentration dependent manner (Fig. 1A). When the concentration was below 5mg/ml, the radical scavenging activities of syringin, polysaccharides and flavones all increased significantly with the increasing concentration. At 5mg/mL, the radical scavenging activities of polysaccharides, flavones, syringin and eleutheroside E were 72.3, 83.0, 58.3 and 13.2% respectively. The IC_{50} values of the extracted polysaccharides, flavones and syringin were 1.4mg/ml, 2.2mg/ml and 4.2mg/ml, respectively. These results demonstrate that, compared with syringin and eleutheroside E, polysaccharides and flavones were more effective in DPPH radical scavenging (Fig. 1A).

In the case of the ABTS assay, the results were similar to those of the DPPH assay. As shown in Fig. 1B, the radical scavenging activities of the active compound extracts increased in a concentration dependent manner. The IC_{50} values of the extracted polysaccharides and flavones were 2.3mg/ml and 1.5mg/ml, respectively. The results show that the polysaccharides and flavone extracts exhibited higher radical scavenging activities than than those of syringin and eleutheroside E. However, these results must be interpreted with some caution as it is possible that a certain amount of impurities may have contributed to the observed antioxidant activities.

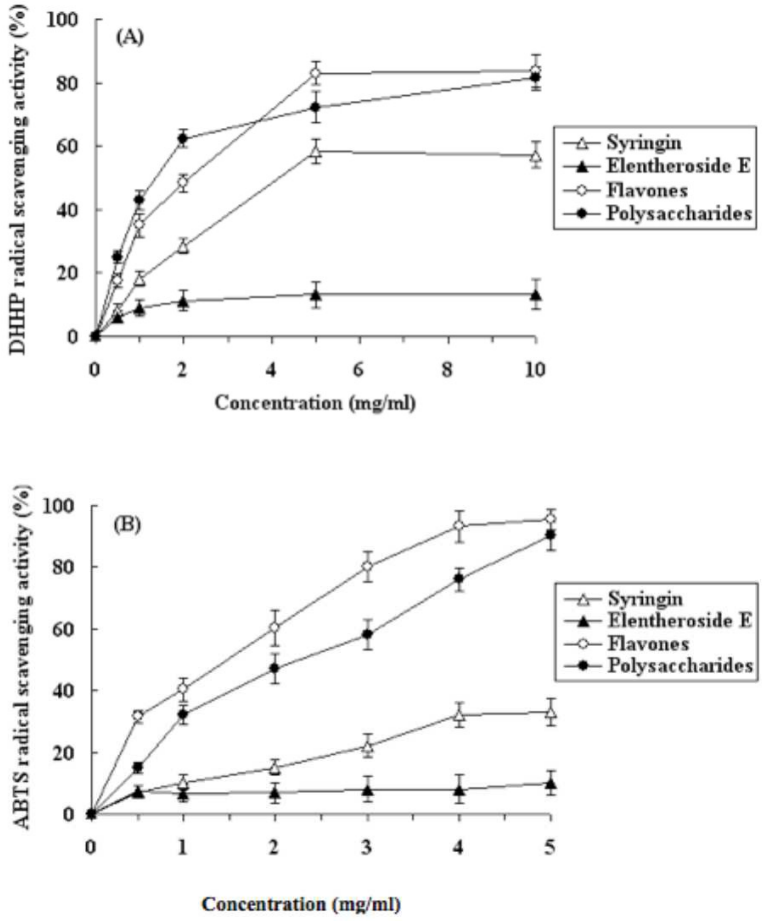

Figure1. DPPH (A) and ABTS (B) radical scavenging activities of polysaccharides, flavones, syringin and eleutheroside E extracted from LAS. Each value is the mean ± SD of triplicate measurements.

3.4. In vivo antioxidant activities of active compounds from LAS

3.4.1. Effects of active compounds from LAS on MDA content in mice after radiation

In order to detect the effects of the active compounds extracted from LAS on radiation induced oxidative stress, the content of MDA in different tissues of the experimental mice (liver, spleen, kidney, testicle and brainstem), was analyzed. As shown in Table 2, compared with the normal control group, radiation induced tissue MDA content was statistically significantly higher in the irradiated control group ($P<0.001$). Among the active compounds extracted from LAS, polysaccharides and flavones statistically significantly decreased the radiation induced MDA levels in all the examined tissues at both low dose (5mg/day) and high dose (10mg/day) levels ($P<0.05$). Eleutheroside E, when administered to the mice at the higher dose level, significantly decreased radiation induced MDA levels in the liver, spleen, brainstem ($P<0.01$) and testicle ($P<0.05$). These results indicate that polysaccharides, flavones and eleutheroside E can exhibit a significant protective effect on heavy ion radiation induced oxidative damage in mice tissues. Moreover, the MDA levels in tissues extracted from the irradiated mice were also lower in the polysaccharides plus flavones group (P+F group in Table 2). This protective effect was more significant in the liver and kidney tissues of the mice.

Table 2. Effects of active compounds from LAS on MDA content in mice after heavy ion radiation

Group	Dose(mg/d)	Content of MDA (nmol/mgprot)				
		Liver	Spleen	Kidney	Brainstem	Testicle
Normal control		6.83±0.72	4.85±0.54	3.75±0.85	3.23±0.41	2.58±0.66
Radiation control		15.98±2.21[a]	10.27±1.96[a]	9.24±2.18[a]	8.33±3.56[a]	6.72±1.13[a]
Polysaccharides	10	7.12±0.85[b]	5.69±1.57[b]	4.10±1.33[b]	4.33±1.44[b]	3.85±1.33[b]
	5	9.31±0.98[b]	7.54±2.13[b]	6.56±2.10[b]	5.45±1.69[b]	4.13±2.13[b]
Flavones	10	7.94±1.17[b]	5.14±1.67[b]	4.66±1.46[b]	4.24±1.30	3.56±1.50[b]
	5	10.19±1.58[c]	8.88±1.20[b]	7.34±2.82[b]	6.56±3.10[b]	4.55±1.66[b]
Eleutheroside E	10	12.98±1.73[c]	9.33±2.22[c]	8.08±2.22	6.46±5.30[c]	5.11±1.33[b]
	5	14.22±2.17	9.91±1.90	8.56±2.14	7.64±4.98	5.89±2.14
Syringin	10	13.43±2.49	9.34±3.60[c]	8.39±2.45	6.58±3.20[c]	5.98±3.23
	5	15.44±3.18	10.90±2.90	9.13±3.68	7.88±4.29	6.65±2.10
P+F*	(3+7)	7.15±0.64[b]	5.09±1.18[b]	4.18±1.37[b]	3.98±1.37[b]	3.44±1.23[b]
	(1.5+3.5)	8.55±0.89[b]	7.14±1.88[b]	5.99±1.83[b]	5.17±1.58[b]	4.24±1.85[b]

* Polysaccharides plus flavones treatment group; [a] P<0.001 (compared with normal control); [b] P<0.05 (compared with radiation control); [c] P<0.01 (compared with radiation control).

3.4.2. Effects of active compounds from LAS on SOD activity in mice after radiation

The activity of SOD in the different tissues of mice (liver, spleen, kidney, brainstem and testicle), was detected in each treatment group. The results are listed in Table 3. Compared with the irradiated control group, mice administered with polysaccharides and flavones showed significantly increased SOD activity in all examined tissues at the high and low dose level. Eleutheroside E and syringin increased the activity of SOD in liver and spleen at high dose (P<0.05).vAt the low dose level (5mg/day), eleutheroside E and syringin did not show statistically significant effects on SOD activity in the kidney. These results demonstrate (in mice), that these active compounds from LAS decrease radiation induced oxidative damage through protection of the enzymes of the antioxidant system.

Table 3. Effects of active compounds from LAS on SOD activity in mice after heavy ion radiation

Group	Dose	SOD activity (U/mgprot)				
		Liver	Spleen	Kidney	Brainstem	Testicle
Normal control		433.30±33.20	262.50±16.25	153.25±7.58	96.45±8.68	100.88±8.30
Radiation control		253.55±19.23[a]	180.60±18.30[a]	88.56±6.53[a]	43.45±6.63[a]	68.54±9.12[a]
Polysaccharides	10mg/d	390.35±18.50[b]	238.20±23.85[b]	123.33±14.41[b]	87.20±7.25[b]	91.78±8.50[b]
	5mg/d	350.45±22.31[b]	205.22±18.33[b]	100.24±9.30[b]	71.33±8.90[b]	86.45±7.12[b]
Flavones	10mg/d	361.45±26.76[b]	255.80±25.30[b]	115.22±13.00[b]	88.79±5.36[b]	85.34±10.10[b]
	5mg/d	328.80±17.20[b]	224.50±20.80[b]	95.25±8.45[c]	72.57±7.75[b]	78.36±6.47[c]
Eleutheroside E	10mg/d	256.19±31.66	218.44±19.33[b]	98.69±13.25	51.47±9.13[c]	74.85±9.70
	5mg/d	225.33±32.12	195.90±14.88	87.44±12.30	46.35±8.53	70.15±9.41
Syringin	10mg/d	280.24±42.20	208.44±20.22[c]	90.77±15.41	47.10±5.39	79.63±7.50
	5mg/d	268.45±48.33	195.52±18.65	92.38±9.78	49.65±9.38	71.44±10.22
P+F*	(3+7)mg/d	411.53±15.80[b]	258.25±22.44[b]	141.05±16.13[b]	89.05±8.20[b]	90.21±8.45[b]
	(1.5+3.5)mg/d	385.45±21.16[b]	235.22±19.64[b]	118.90±12.55[b]	77.59±9.90[b]	85.60±9.34[b]

* Polysaccharides plus flavones treatment group; [a] P<0.001 (compared with normal control); [b] P<0.05 (compared with radiation control); [c] P<0.01 (compared with radiation control).

3.5. Effects of active compounds from LAS on proteins in mice liver after radiation

Mouse liver proteins were separated by 2-DE. A total of 18 gels (3 individuals × 6 treatment groups) were produced. The 6 treatment groups included: normal control group; irradiated control group; polysaccharide (10mg) treatment group; flavone (10mg) treatment group; eleutheroside E (10mg) treatment group; syringin polysaccharides and flavones (10mg, 3mg and 7mg respectively) treatment group. Approximately 300 spots in each gel were detected by the automated spot detection algorithm across the 18 gels. Proteins associated with each gel spot were identified by MALDI mass spectrometry analysis. Three proteins including heat shock protein 90B1 (HSP90B1), disulfide-isomerase A6 (PDIA6) and glutathione S-transferase pi (GSTP1) were identified as the significantly changed spots. The expression levels of the above 3 proteins in each treatment group were compared in Fig. 2.

As shown in Fig. 2, the expression levels of proteins HSP90B1, PDIA6 and GSTP1 were significantly higher after radiation treatment. In the polysaccharides and flavones treatment groups, the expression levels of the three detected proteins were lower than those of the radiation controls. Protein HSP90B1 is a member of the Hsp90 family which is involved in maintaining protein homeostasis in the cell secretory pathway as well as functioning in the intracellular trafficking of peptides from the extracellular space to the MHC class I antigen processing pathway of antigen presentation cells. HSP90B1 plays a key role in signal transduction, protein folding and protein degradation. Protein PDIA6 belongs to the protein disulfide isomerase family and is found in the endoplasmic reticulum where it catalyzes the formation and breakage of disulfide bonds between cysteine residues within proteins as they fold. Protein GSTP1 plays a pivotal role in the detoxification of xenobiotics, as well as in carcinogenesis and drug resistance. Our data have demonstrated that polysaccharides and flavones extracted from LAS significantly repressed the expression changes of these detected proteins induced by heavy ion radiation. Not with standing any activities that may have been caused by impurities in the extracted samples, these results suggest that polysaccharides and flavones derived from LAS could significantly decrease radiation-induced damage in the livers of mice.

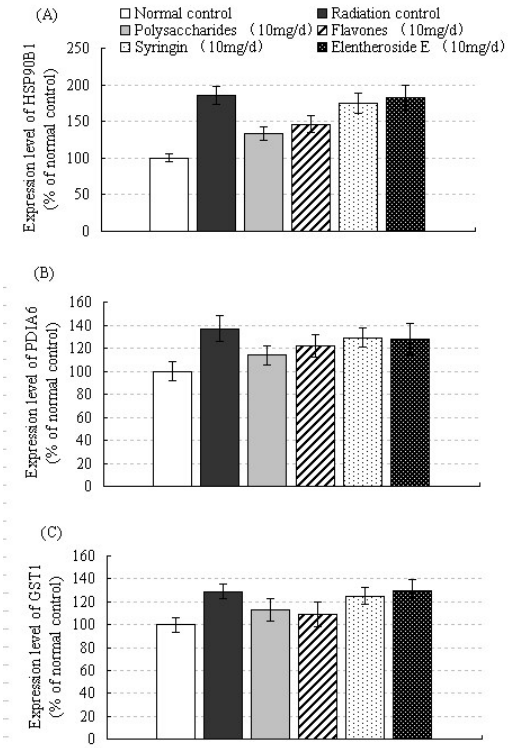

Figure 2. Expression level of HSP90B1, PDIA6 and GSTP1 proteins of mice liver (A) The expression of HSP90B1 with active compounds treatment. (B) The PDIA6 protein expression after active compounds treatment. (C) The GSTP1 protein expression after compounds treatment

4. Conclusions

This study has indicated that polysaccharides and flavones extracted from wild *Acanthopanax senticosus* from the Lesser Khingan Mountain range in China, could be radioprotective agents against ion-radiation induced oxidative damage in animal tissues. These data provide a new perspective on human radiation protection and are potentially of relevance to the protection of humans in occupations involving specialist types of work where there is radiation exposure. Also in our study, we observed that three different kinds of proteins extracted from mice livers were down regulated after mice were administered (through diet), with extracts of the active components derived from *Acanthopanax senticosus*. Further studies will need to focus on identifying the precise mechanism of regulation of these proteins during the anti-radiation response.

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Author Contributions

Weihong Lu conceived this study; Wei Song wrote the paper; Jinming Shi conducted the experiments; Denis Baranenko and Jing Jing analyzed the data.

Conflicts of Interest

The authors declare no conflict of interest.

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