Influence of Different Extraction and Purification Methods on Astragalus Polysaccharides and Pharmacological Evaluation

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Abstract: **Objective** To clarify the influence on component and pharmacological action of Astragalus polysaccharides (APS) as complementary therapeutic agents prepared by different extraction and purification techniques. **Methods** Components of APS prepared by different extraction and purification techniques were analyzed, and these APS were used for synergy and attenuation of chemotherapy, radiotherapy treatment with H_{22} liver cancer and Lewis lung cancer of tumor-bearing mice, and also used for the regulation of immune function to immunosuppression mice. **Results** Experimental data were analyzed by means of statistical method to get pharmaco-result: A3 (extracted by microwave assistance and purified by membrane separation) > A4 (extracted by refluxing and purified by membrane separation) > A1 (extracted by refluxing and no purification) ≈ A2 (extracted by microwave assistance and no purification). There were no significant differences on pharmacodynamic action between A1 and A2. However, compared with A1 and A2, it was worth noting that A3 and A4 exhibited good pharmacodynamic action. Then A3-in and A4-in, the samples in dialyzer after dialysis, were separated and purified to get homogeneous APS, which were the principal constituents of APS in dialyzer, with the molecular weight (*M*w) of 7669 and 14 142 determined by HPGPC, respectively. The average *M*w of APS outside of the dialyzer, A3-out was 3102 and A4-out 3256, which were the main compositions of A3 and A4, accounted for 79.63% and 53.92%, respectively. **Conclusion** APS with *M*w about 5000 Da exhibit better antitumor effect and immunological activity. Refluxing, microwave assistance extractions, and membrane enrichment techniques bring different cases on *M*w distribution, components and pharmacodynamic action, and obviously exhibit relationship among component, *M*w distribution, and pharmacological action.

Key words: Astragalus polysaccharides; component; extraction and purification methods; molecular weight distribution; pharmacodynamic action

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Introduction

Astragalus polysaccharides (APS), the main functional components of *Radix Astragalus*, were reported to exhibit diverse bioactivities including immunoregulation, effects of anti-tumor, radio-protection, anti-hypoxia, hepatic, and cardiovascular system protection (Zhu *et al*, 1998). However, research on component and immunoregulation, synergy and amelioration of chemotherapy, radiotherapy treatment on tumor-bearing mice with APS prepared by microwave assistance extraction, and study on effects of different extraction and purification methods on pharmacodynamic action, component of APS, and the correlation between them were carried out.

To the best of our knowledge, there is still no experiment data reported. In addition, different extraction and purification methods of APS were designated, the components of APS prepared from different extraction and purification methods were analyzed, and pharmacological experiments of APS were evaluated by spleen, thymus, WBC count, tumor inhibition rate, and immunoregulation index. The results showed that APS prepared from different extraction and purification methods were different

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in both physicochemical properties and pharmaco-result. These results suggested that it was necessary to explore the relationship and the initial mechanism, which may help to establish the foundation of elucidating material basis of pharmacodynamic action and developing of new drug.

Materials and methods

Apparatus and chromatographic materials

Microwave assistance oven specially for experiment model: NJL073 (Jiequan Co., Ltd., Nanjing, China); UV-VIS 8500 (Tianmei Equipment Co., Ltd., Shanghai, China); Ultrafiltrate bowl for experiment (Yadong Co., Ltd., Shanghai, China); Ceramic membrane (Hydro Air Research Corporation, Italy); Ultrafiltration membrane (HAR, Italy); Bag filter MWCO3500, MD-25 (Lvniao Co., Ltd., Shanghai, China, Batch No. 20050820); DEAE-cellouse–DE-52 anion exchange resin (Whatman, United Kingdom, Batch No. 6152040); Sephadex G-100 (Pharmacia, Sweden, Batch No. 308579); BT01-100 Constant flow pump YZ1515 Lan Ge PUMP (Baoding, China); Gel chromatographic column: Bio-sep-sec S3000, Guard column matching product 35 mm × 7.8 mm (Phenomenex, USA), Waters HPLC515 (Waters, USA), Waters2410 RID, GPC data processing system of Minennium 32 edition; Hitachi H-600 transmission electron microscope (Hitachi, Japan).

Drugs and standard substance

Astragalus membranaceus (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao was collected in September 2005, in Hunyuan, Shanxi, China, identified as root of *A. membranaceus* var. *mongholicus* by Prof. WU Tong (Shanghai Institute of Pharmaceutical Industry) and a voucher specimen (AM 05-09) was preserved in our laboratory; Cellulase (SCRC 64001131, Batch No. F 20040719, activity unit ≥ 1000 U/g); Polysaccharides standard substance-Dextran D bought from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Batch No. D1 140638-200001, D2 140639-200001, D3 140640-200001, D4 140641-200001, D5 140642-200001, D6 140643-200001, D7 140644-200001, D8 140645-200001); Cyclophosphamide (CTX) (Hengrui, Co., Ltd., Jiangsu, Batch No. 05122921); Diamminedichloroplatinum (DDP) (Qilu Co., Ltd., Shandong, Batch No. 050717); Hydrocortisone (HY) (Xinyi Co., Ltd., Shanghai, Batch No. 050902); A1, A2, A3, A4 are prepared by the experimenters.

Agents

All the agents used in the experiment are analytical reagents, made in China.

Experimental animals

Kunming Mus musculus albus, C57BL/6J Mus rattus. bought from Sipuar-Bkay Laboratory Animals Co., Ltd., Shanghai, China.

Results

Comparison of different extracting methods

Raw materials of *Radix Astragali* were extracted by five different methods: reflux, potass, microwave assistance, ultrasound, and enzymic method. The methods were compared and optimized with the parameters of extraction ratio and purity of APS. The results are presented in Table 1.

Table 1 Comparison of different extracting methods

Extracting method	Purity / %	Extraction yield / %
Microwave assistance	61.34	12.98
Refluxing	45.64	9.12
Potass method	40.34	7.37
Enzymic method	40.18	6.76
Ultrasound	24.86	1.72

The extraction yield and purity of APS with microwave assistance were both obviously higher than those of other four methods, and the following one was refluxing method. In addition, the microwave assistance extraction time was 6–7 times shorter than that of refluxing, so microwave assistance extraction was found to be the optimal extraction method for practical significance. And as a traditional method, refluxing is extensively applied in extracting APS. So comparison of the APS obtained by reflux with that by microwave assistance has significant meaning.

Preparation of samples by different techniques

Radix Astragali was broken into powdered samples and the average particle diameter of powdered *Radix Astragali* has been fixed. Particles with an average diameter of (850 ± 29) μm have been always used. With raw materials of *Radix Astragali*, extracted by microwave assistance, micro-filtered by ceramics membrane, the liquid was then ultrafiltrated by NF-TCM organ-membrane, precipitated by ethanol, with freeze

drying, then A3 was got. And the part that wasn't filtered but precipitated by ethanol directly was A2; The part that was extracted by refluxing, micro-filtered by ceramics membrane, the liquid was then ultrafiltrated by NF-TCM organ-membrane, precipitated by ethanol, with freeze drying was A4; And the part that wasn't filtered but precipitated by ethanol was A1. The APS contents in A1, A2, A3, and A4 were evaluated to be 50.01%, 59.14%, 85.74%, and 78.80%, respectively.

Comparison of A3 and A4 after dialysis

Due to the molecular weight (*M*w) of polysaccharide influencing the pharmacodynamic action and the necessity of dialysis before separation and purification of polysaccharide, the dialyzer with the *M*w 3500 was chosen to deal with A3 and A4, after vacuum drying, and two parts were obtained: the APS in dialyzer of *M*w > 3500, named A3-in and A4-in, and that outside of dialyzer, *M*w < 3500 named A3-out and A4-out. Each of them was detected by HPGPC respectively to get information of *M*w (Zhao *et al*, 2000). The results showed that APS with different extraction methods had difference in *M*w characterization.

Comparison of samples in dialyzer after dialysis A3-in, 20.33% content in A3, was pale yellow and freely soluble in water. A4-in, 46.08% content in A4, was pale white and slightly soluble in water. HPGPC chromatograms were referred to Fig. 1.

A was divided into 4 parts, A1 (15–20 min), A2 (20–25 min), A3 (25–38 min), A4 (38–43 min) and the same occurred on B. When A was compared with B, there was more B1 and almost no A1; more A2 than B2; A3 and B3 were the most contents respectively in A and B and B4 was more than A4, A4 almost couldn't be found. With extraction methods being considered, a conclusion that the microwave assistance method might transfer A1 into A2 and then A3 can be made.

Comparison of samples outside dialyzer after dialysis

A3-out, 79.67% content in A3, was yellow and freely soluble in water. A4-out, 53.92% content in A4, was pale yellow and freely soluble in water. HPGPC chromatograms were referred to Fig. 1.

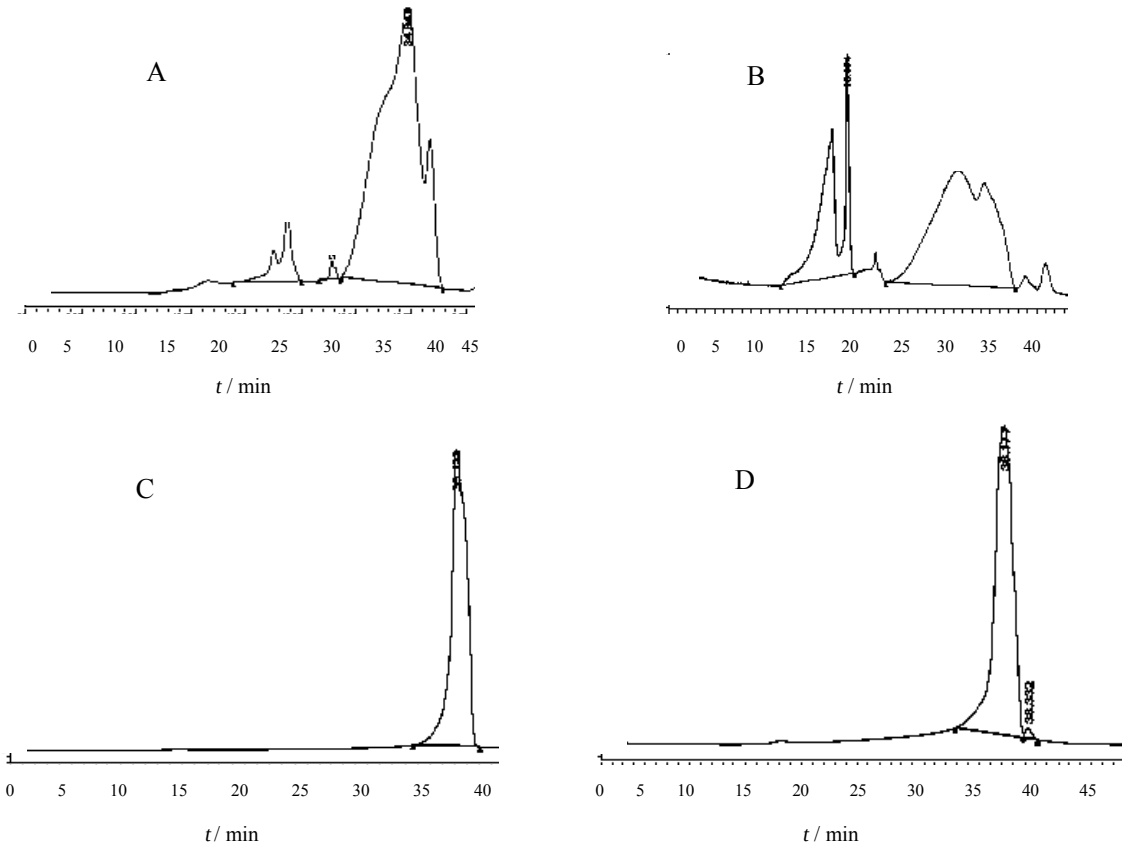

Fig. 1 Comparison of HPGPC chromatograms of A3-in (A), A4-in (B), A3-out (C), and A4-out (D) after dialysis

Comparing C with D, the chromatograms of C, C3-out and D C4-out, are almost the same, the retention time (t_R) and average *M*w of polycose with micro-molecule outside dialyzer were almost the same as well.

Proportion and *M*w of samples

Proportion and *M*w of samples are in Table 2.

Comparison of chromatographic separation behaviors using DEAE-cellouse column

DEAE-cellouse column chromatography was applied for prefractionation, eluted by water and sodium chloride with 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, and 1.5 mol/L, collected in different levels and *M*w was determined by HPGPC. It has been calculated that yield = weight of obtained sample / total weight × 100%.

Table 2 Proportion and *M*w of samples

Range	Average t_R / min	Average *M*w / $\times 10^4$	Proportion / %	Average *M*w in dialyzer / $\times 10^4$	Proportion in dialyzer / %	Average *M*w outside dialyzer / $\times 10^4$	Proportion outside dialyzer / %	Total average *M*w
A1	17.159	40.13	2.10					
A2	24.206	8.97	18.95	1.32	20.33	0.31	79.67	0.51
A3	35.324	0.72	77.68					
A4	41.311	0.25	1.27					
B1	17.115	40.67	21.50					
B2	23.303	30.30	14.00	13.03	46.08	0.32	53.92	6.18
B3	32.124	1.47	61.09					
B4	42.172	0.16	3.41					

Fig. 2 showed that the trend curves of A3-in and A4-in were almost the same, along with rising of salt concentration, *M*w of eluted APS increased at first and then decreased and the yield decreased in the same order, so the part eluted by water accounted most of the APS, and named A3-in-DEAE-W and A4-in-DEAE-W. But the part eluted by 0.5 mol/L NaCl of A3-in had the largest *M*w of appears at Part E, and the part of A4-in, which has the largest *M*w, was eluted by 0.2 mol/L NaCl appears at Part C.

Comparison of samples crossing Sephadex G-100 column chromatography

A3-in-DEAE-W and A4-in-DEAE-W respectively accounted 63.52%, 75.32% of total A3-in-DEAE and A4-in-DEAE were separated and purified through Sephadex G-100 column chromatography, and the elution curve and HPGPC chromatograms of each sample were compared.

Comparison of samples through Sephadex G-100 column chromatogram (normal pressure column chromatography) By contrasting the results showed in Fig. 3, it was obvious that the trends in chromatograms of samples were almost the same, the peak of A3-in-DEAE-W appeared at 40 mL point, and eluted completely at 64 mL point, the one of A4-in-DEAE-W appeared at 50 mL, and eluted completely at 88 mL. The peak amplitudes were almost the same, but the chromatogram of A3-in-DEAE-W had a better symmetry over A4-in-DEAE-W.

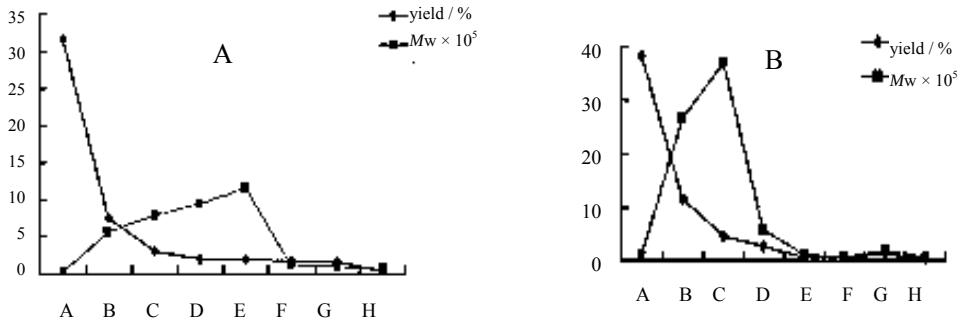

Fig. 2 Comparison of yield and *M*w of samples after DEAE–cellouse (A: A3-in-DEAE, B: A4-in-DEAE)

A, B, C, D, E, F, G, and H represent the part that eluted by water, 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, and 1.5 mol/L NaCl, respectively

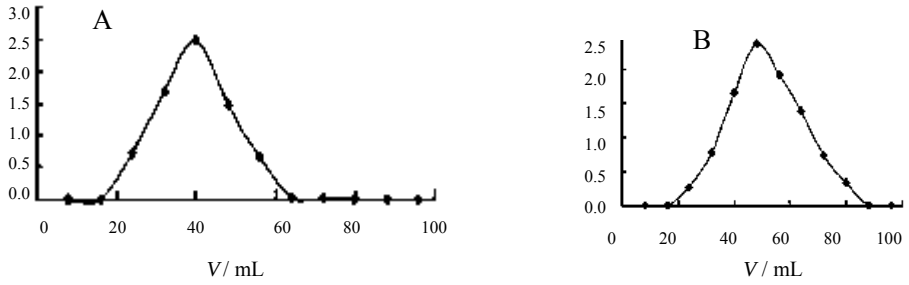

Fig. 3 Comparison of elution curves of APS through Sephadex G-100 (A: A3-in-DEAE-W, B: A4-in-DEAE-W)

Comparison of samples through Sephadex G-100 detected by HPGPC (high pressure column chromatography) According to the curves showed in Fig. 4, the *M*w of A3-in-DEAE-W-G and A4-in-DEAE-W-G were estimated by HPGPC to be 7669 and 14 142, and content was 96.32% and 95.67%, with each t_R 34.748 and 31.731 min, respectively. They really had difference on *M*w (Jiang *et al*, 2005).

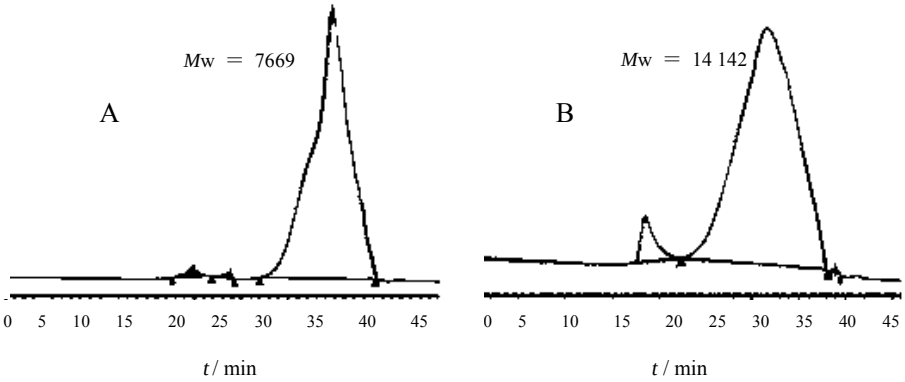

Fig. 4 Comparison of HPGPC graph of APS after Sephadex G-100 (A: A3-in-DEAE-W-G, B: A4-in-DEAE-W-G)

Pharmacodynamic actions of APS A1, A2, A3, and A4 were observed by pharmaco-experiments of synergy and amelioration of chemotherapy, radiotherapy treatment with H_{22} liver cancer and Lewis lung cancer of tumor-bearing mice and for the regulation of immune function to immunosuppression mice (Ding *et a1*, 2005; Yuan, Chen, and Yan, 2005).

Synergy and amelioration of APS towards H_{22} tumor-bearing mice treated with CTX

Mice were randomly divided into six groups: a normal control group, a model control group, an A1 treatment group, an A2 treatment group, an A3 treatment group, and an A4 treatment group. H_{22} liver cancer cells were implanted into the six groups by sc injection at cell concentration of 2×10^6/mL. The mice in the four treatment groups received ig administration of A1, A2, A3, A4, at dosages showed in Table 3 from the second day, respectively. The dosage is 8 g crude herbal per 1 kg mice according to the criterion of equal crude herbal dosage covert (the same below).

At the same time, the mice of model control group were given CTX by ip injection according to a dose of 30 mg/kg. The mice were observed by general condition everyday. The mice were totally administered for 12 d. WBC count was analyzed in blood sample obtained from iv injection 2 h after the last administration. After corresponding administration, the mice were sacrificed and the tumor, spleen, and thymus were cut and weighed immediately to calculate the suppression rate. Results are shown in Table 3.

Synergy and amelioration of APS towards Lewis tumor-bearing mice treated with DDP

Mice were randomly divided into six groups: a normal control group, a model control group, an A1 treatment group, an A2 treatment group, an A3 treatment group, and an A4 treatment group. Lewis lung cancer cells were implanted into six groups by sc injection at cell concentration of 2×10^6/mL. The mice in the four treatment groups received ig administration of A1, A2, A3, and A4 with dosages showed in Table 3 from the

Table 3 Synergy and amelioration of samples toward H_{22} tumor-bearing mice treated with CTX (n = 6, $\bar{x} \pm s$)

Group	Dosage / ($g \cdot kg^{-1}$)	CTX / ($mg \cdot kg^{-1}$)	Spleen index / ($g \cdot kg^{-1}$)	Thymus index / ($g \cdot kg^{-1}$)	WBC number / ($\times 10^9 \cdot L^{-1}$)	Tumor weight / g	Tumor restrain ratio / %
A1	1.2472	30	10.78 ± 3.81△	2.76 ± 1.00	11.74 ± 4.45△	1.10 ± 0.64▲▲	43.74
A2	1.0088	30	8.87 ± 3.18	3.16 ± 1.73	9.76 ± 3.97	0.84 ± 0.49▲▲	57.21
A3	0.6520	30	10.45 ± 4.01△	2.76 ± 0.64	11.53 ± 5.63△	0.78 ± 0.75▲▲	60.37
A4	0.5120	30	8.91 ± 2.30	3.13 ± 1.52	7.55 ± 2.84	0.61 ± 0.47▲▲	68.84
CTX	-	30	7.00 ± 3.16	2.39 ± 0.69	7.95 ± 3.21	1.00 ± 0.43▲▲	57.49
Model	-	-	10.09 ± 3.98	2.96 ± 1.25	10.07 ± 3.07	1.95 ± 1.19	—

△ $P < 0.05$ *vs* CTX group; ▲▲ $P < 0.01$ *vs* model group

second day, respectively. At the same time, the mice of model control group were given DDP by ip injection with a dose of 3 mg/kg. The following way was the same with described before. Results are shown in Table 4.

Synergy and amelioration of samples towards H_{22} tumor-bearing mice treated with ^{60}Co ray

Mice were randomly divided into six groups: a normal control group, a model control group, an A1 treatment group, an A2 treatment group, an A3 treatment group, and an A4 treatment group. Lewis lung cancer cells were implanted into six groups by sc injection at concentration of 2×10^6/mL. The mice in the four treatment groups received ig administration of A1, A2, A3, and A4 at dosages showed in Table 3 from the second day, respectively. On the 5th and 10th day, the mice of model control group were exposed to ^{60}Co-gamma ray at a dose rate of 0.55 Gy/min till dose arrival at 5Gy each time. The following way was the same with described before. Results are shown in Table 5.

Table 4 Synergy and amelioration of APS towards Lewis tumor-bearing mice treated with DDP (n = 6, $\bar{x} \pm s$)

Group	Dosage / ($g \cdot kg^{-1}$)	DDP / ($mg \cdot kg^{-1}$)	Spleen index / ($g \cdot kg^{-1}$)	Thymus index / ($g \cdot kg^{-1}$)	WBC number / ($\times 10^9 \cdot L^{-1}$)	Tumor weight / g	Tumor restrain ratio / %
A1	1.2472	3	3.53 ± 2.43▲▲	2.26 ± 1.71	7.09 ± 2.75▲▲	1.41 ± 0.58▲▲	44.09
A2	1.0088	3	3.71 ± 2.11▲▲	1.94 ± 0.67	9.82 ± 3.11	1.47 ± 0.49▲▲	41.66
A3	0.6520	3	6.71 ± 2.80△▲▲	1.42 ± 0.99▲	9.63 ± 3.83	1.03 ± 0.32▲▲	59.14
A4	0.5120	3	4.70 ± 2.27▲▲	2.34 ± 1.44	7.51 ± 2.39▲▲	1.07 ± 0.38▲▲	57.79
DDP	-	3	3.66 ± 0.81▲▲	2.02 ± 0.80	7.34 ± 3.67▲▲	1.45 ± 0.44▲▲	42.56
Model	-	-	10.35 ± 3.28△△	2.76 ± 1.05	12.91±3.66△△	2.52 ± 0.59	—

△ $P < 0.05$, △△ $P < 0.01$ *vs* DDP group
▲ $P < 0.05$, ▲▲ $P < 0.01$ *vs* model group

Table 5 Synergy and amelioration of samples towards H_{22} tumor-bearing mice treated with ^{60}Co ray (n = 6, $\bar{x} \pm s$)

Group	Dosage / ($g \cdot kg^{-1}$)	^{60}Co / Gy	Spleen index / ($g \cdot kg^{-1}$)	Thymus index / ($g \cdot kg^{-1}$)	WBC number / ($\times 10^9 \cdot L^{-1}$)	Tumor weight / g	Tumor restrain ratio / %
A1	1.2472	5	9.22 ± 5.10	1.94 ± 0.99	5.29 ± 2.18▲▲	1.68 ± 1.44	34.91
A2	1.0088	5	8.80 ± 3.00	1.42 ± 0.89	5.72 ± 3.63▲▲	1.97 ± 1.11	23.61
A3	0.6520	5	9.41 ± 4.76	1.53 ± 0.99	8.97 ± 4.74△△	1.33 ± 0.77▲	48.27
A4	0.5120	5	9.66 ± 2.78	1.57 ± 0.54	7.25 ± 2.40▲	1.54 ± 0.68▲	40.39
RAD	—	5	6.75 ± 2.94▲▲	1.02 ± 0.55▲	4.98 ± 3.12▲▲	2.04 ± 0.72	20.81
Model	—	—	12.32 ± 8.58△	2.39 ± 1.51△	10.34 ± 2.11△△	2.57 ± 1.18	—

△ $P < 0.05$, △△ $P < 0.01$ *vs* RAD group
▲ $P < 0.05$, ▲▲ $P < 0.01$ *vs* model group

immunity suppression animal model

Mice were randomly divided into five groups: a model control group, an A1 treatment group, an A2 treatment group, an A3 treatment group, and an A4 treatment group. HY were given into five groups by ig at the dosage of 30 mg/kg. The mice in the four treatment groups received ig administration of A1, A2, A3, and A4 at dosages showed in Table 5 from the second day, respectively. WBC count was analyzed in blood sample obtained from vena iv from the second day. The following way was the same with described before. Results are shown in Table 6.

Analysis of experimental data

Table 6 Regulation of immune function of samples towards immunity suppression animal model (n = 6, $\bar{x} \pm s$)

Groups	Dosage / ($g \cdot kg^{-1}$)	HY / ($mg \cdot kg^{-1}$)	Spleen index / ($g \cdot kg^{-1}$)	Thymus index / ($g \cdot kg^{-1}$)	WBC number before model / ($\times 10^9 \cdot L^{-1}$)	WBC number after model / ($\times 10^9 \cdot L^{-1}$)
A_1	1.2472	30	3.78 ± 2.24	2.06 ± 0.92	11.90 ± 5.01	8.94 ± 3.81▲
A_2	1.0088	30	4.10 ± 2.34	1.97 ± 0.51	13.30 ± 5.28	8.30 ± 2.86
A_3	0.6520	30	6.40 ± 4.59▲▲	2.79 ± 0.82▲▲	12.41 ± 3.72	11.10 ± 3.82▲▲
A_4	0.5120	30	3.86 ± 0.83	3.72 ± 0.71▲▲	12.10 ± 2.10	8.39 ± 3.80
Model	-	30	3.08 ± 1.50	1.56 ± 0.33	12.72 ± 1.89	5.67 ± 2.00

▲ $P < 0.05$, ▲▲ $P < 0.01$ *vs* model group

A new method which was standardized deviate and maximization of mean difference was applied to determine pharmaco-evaluation, the results are as follows. Table 7 shows that A3 is better than A4 (Wang and Zhang, 2003).

Table 7 Results of every sample

Groups	CTX	DDP	RAD	HY	Total
A1	40.13	12.25	43.75	35.40	30.56
A2	42.61	20.13	25.31	32.72	29.43
A3	50.10	63.33	69.52	84.79	76.42
A4	42.42	46.00	53.91	59.78	55.58

Discussion

The extraction yield and purity of polysaccharides extracted by microwave assistance were quite high maybe because of its selectivity for polysaccharides.

That pharmacodynamic action of A3 was better than A4 after ig administration could be interpreted as follows: It is more difficult for APS with high *M*w to permeate biofilm and get into tissue and organ. APS extracted by refluxing mainly are component of a high *M*w, so APS with *M*w of about 400 000 account for 16.35% in A4; APS extracted by microwave assistance mainly are component of a low *M*w, so APS with *M*w of about 5000 account for 98.54% in A3. Microwave assistance cavitation effect made APS with high *M*w transforming into APS with low *M*w results in difference in *M*w characterization and t_R between the two samples.

A3 with the average *M*w of 5146 is lower than A4 with the average *M*w of 61 380, so a conclusion can be made easily: *M*w has certain relationship with pharmacodynamic action, the *M*w of APS extracted by microwave assistance was exactly appropriate, whose pharmacodynamic action was better. It can be concluded that the difference in pharmacodynamic action is to be reflected in the small *M*w part. During the microwave assistance extraction, molecular structure of APS may be changed, or get structural modification, fragment structure was lost, or reconstructed and recombined on other small fragment structure. All the above may increase the pharmacodynamic action of APS, the mechanism was under being researched.

Pharmacodynamic action of crude extract of A1 and A2 had no obvious difference, both two were not good and may be related to a lot impurity in crude extract, affected the pharmacodynamic action of APS, but after filtered and purified through membrane, to get A3 and A4 whose pharmacodynamic action obviously increased. It proved once again, APS with the suitable *M*w, whose pharmacodynamic action was better. It also indicated purity may have an effect on pharmacodynamic action, so it illustrated that extraction and enriching technologies used in Chinese materia medica can obviously affect the pharmacodynamic action further.

The part eluted by water through DEAE-cellouse column chromatography was the most, A3-in and A4-in account for 63.52% and 75.32% of the product in dialyzer, respectively. And then separated and purified to get two purified APS, and the purity and *M*w were estimated by HPGPC to be 96.32%, 7669 and 95.67%,

14 142, respectively, which is the principal constituent of APS in A3 and A4. Difference in constituents of APS with small *M*w may also affect pharmacodynamic action.

The *M*w of APS outside of dialyzer: A3 to be 3102, A4 3256, the constituents are fairly pure, the most in A3 and A4, account for 79.67% and 53.92%. The chromatography behaviors are almost the same, and may indicate structure fragment or micromolecule compounds of their APS outside of dialyzer were similar or the same.

Refluxing, microwave assistance extraction and membrane enriching or not exert a great influence on component, *M*w distribution and pharmacodynamic action of APS, and obviously exhibit relationship among component, *M*w distribution and pharmacological action. Physical property, purity, and pharmacodynamic action of APS extracted by microwave assistance are better than those of APS extracted by refluxing. And the pharmacodynamic action of APS that hasn't been purified through membrane is not as good as that has been purified.

To the best of our knowledge, there is still no experiment data reported on pharmacodynamic action of APS prepared by microwave assistance extraction. However, two problems need further research: One is to clarify the influence of extraction methods on APS structure, and the other is to clarify the structure-activity relationship of APS. We also will conduct such research in the future.

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