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Studies on Immunopotentiating Activities of Antitumor Polysaccharide from Aerial Parts of *Taraxacum platycarpum*

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Abstract □ The polysaccharide fraction from Taraxii Herba showed potent immunopotentiating activities with antitumor activities. The fraction having small amount of protein inhibited the growth of solid tumor and increased peritoneal exudate cells and immunoorgan weights in normal mice, and also increased hypersensitivities in tumor bearing mice.

Keywords □ Polysaccharide, antitumor activity, immunopotentiation

Dandelion, *Taraxacum platycarpum* Dahlst (Compositae), is one of the most common potherb or weed throughout the world. The aerial part of the plant, Taraxii Herba, has long been used as a component of prescriptions for abscess, bruises, cancer, caries, catarrh, cholecystosis, eczema, gastritis, gout, hemorrhoid, breast cancer, petence, jaundice and other related internal injuries in oriental and occidental regions.[1,2] It is reported to contain several sterols, organic acids and sugars, and cholin.[3] In order to find out the characteristics of immunopotentiation with antitumor activity of the herb drug, several antitumor and immune activities were checked against polysaccharide fraction as in many previous reports.[4-7]

EXPERIMENTAL METHODS

G.R. grade chemicals were used all throughout the experiments. UV and IR spectra and gas chromatogram were taken with Shimadzu UV-240 and Shimadzu IR-400, and Shimadzu GC-RIA, respectively. Healthy male ICR mice weighting 18-22g were used in animal experiments. Sarcoma 180 tumor cell strain was kindly donated from Dr. C.K. Moon of Seoul National University and cultivated in our laboratory.

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Extraction of polysaccharides

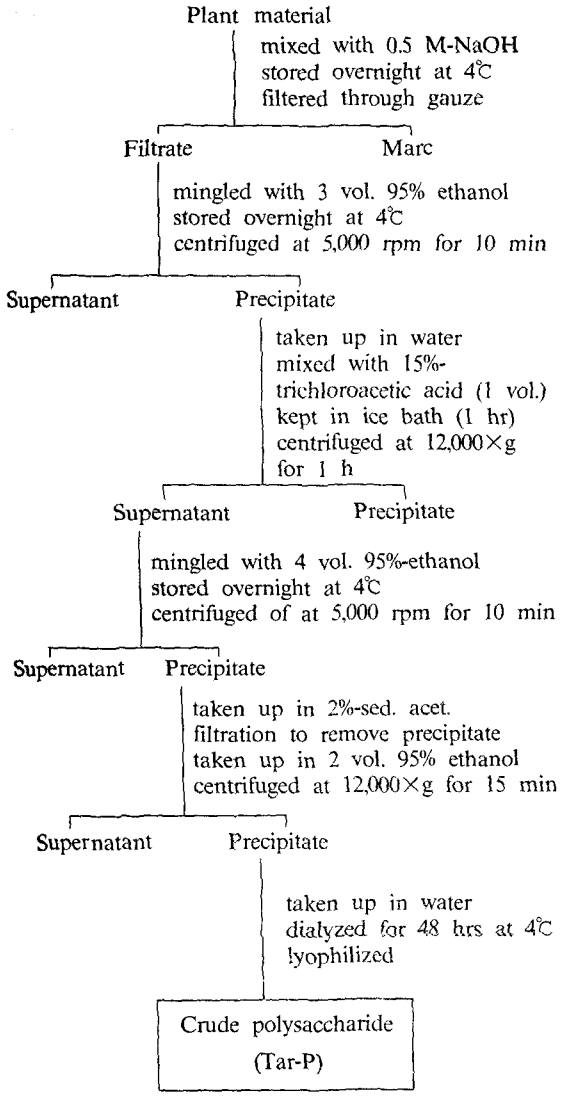
Aerial parts of the herb drug were purchased in a local market and cut into adequate size to extract the polysaccharides (Tar-P) according to the method used by Moon, *et al.*[7] as shown in Scheme 1.

Chemical analyses of components

Polysaccharide content of the fraction was determined by anthrone test of Herbert[8] and monosaccharide constituents were analyzed by gas chromatographic method of Mitruka using methylsilanization.[9] The protein content was determined by Lowry-Folin method with bovine serum albumin (Sigma Fr. V) as a standard[10] and amino acid constituents were analyzed by autoanalyzer. The IR spectra were obtained by KBr disc method.

Antitumor activity test

Mice were implanted subcutaneously into the left groin and intraperitoneally with 1.0×10^6 tumor cells in order to determine effects on solid tumor and life span of mice, respectively.[11] After 24 hrs of inoculation of tumor cells, sample was administered intraperitoneally for 10 consecutive days. Inhibition ratios of solid tumor growth ware calculated from changes in weight after 26 days and prolongation ratio of life span was checked 35 days after the inoculation, respectively. In order to test the direct cytocidal action of the polysaccharide, 2.0×10^5 tu-



Scheme 1. Procedure for extraction of water soluble polysaccharide from arial part of *Taraxacum platycarpum*.

mor cells were incubated with the sample in Eagle's minimal essential medium containing 10%-fetal calf serum using a CO_2 incubator at 37℃ for 24 hrs. Then, cells were stained with trypan blue to estimate their viability 24 hrs later.[12-14]

Immune function test

Peritoneal exudate cells: Twelve animals were injected with the sample for three consecutive days and four animals in each group were sacrificed by cervical dislocation on 1st, 2nd and 4th days after the final administration. The peritoneal exudate cells were collected by peritoneal lavage with 5 ml of saline. Then number of total peritoneal exudate cells were counted in a haemacytometer by using Turk's solution.[15]

Immunoorgan weight: Animals were injected with sample intraperitoneally for 10 consecutive days and liver, spleen and thymus were extirpated from animals sacrificed by cervical dislocation on 8th day from final sample administration. The organ weights of sample treated group were compared with those of control group, which were injected saline only.[16]

Arthus reaction and delayed-type hypersensitivity: Animals were inoculated with 1.0×10^6 tumor cells subcutaneously into left groin (day 0); then the animals were sensitized by injecting 40 μg of bovine serum albumin(BSA, Sigma Fr IV) in 50 μl of emulsified Freund's complete adjuvant or saline subcutanously at tail base on day 2. Sample or saline treatments were followed 24 hrs later (day 1) for 10 consecutive days intraperitoneally. On day 9 each group was challenged with 2%-aggregated BSA (30 μl) at the left footpad and the thickness of footpad was measured 3 hrs (Arthus reaction) and 24 hrs (delayed-type hypersensitivity) later, respectively.[17-20]

Total serum protein Sample was injected intraperitoneally to animals for 10 days then blood was collected from carotid artery two days later after the final administration of sample. Serum was obtained by centrifugation (3,000 rpm, 10 min) to determine the protein content by Biuret method with BSA as a standard.[21]

RESULTS AND DISCUSSION

***Chemical composition of the polysaccharide (Tar P)*[22,23]**

The dialyzed and subsequently lyophillized product from 300g of the aerial part of *Taraxacum platycarpum* was 4.76g of polysaccharide with 45.37% purity. The purity was only determined on the basis of glucose, so the practical polysaccharide purity is considered to be higher. The main impurity was found as protein (6.03%) due to the incomplete removal by trichloroacetic acid. But the differences between partial presence and complete removal of protein component should be further studied as compared with the earlier report which concluded that the presence of protein increased the antitumor ac-

Table I. Antitumor activities of Tar-P against sarcoma-180 solid type and ascites tumors.

Treatments	Dose (mg/kg)	Tumor weight (g)	Average survival days	Inhibition ratio (%)
Control	-	5.92± 0.32	14	
Tar-P	5	4.85± 0.26		18.07*
	10	3.55± 0.13		40.03*
	30	1.55± 0.17	16	73.82**

Data are mean value with standard error from eight animals.
Significance: *, non-significant and **, $p<0.05$.

Table II. Effect of Tar-P on the number of peritoneal exudate cells in male ICR mice (1.0×10^6 cells/ml)

Treatments*	Cell number (mean± S.E.)		
	Day 1	Day 2	Day 4
Control	0.89± 0.09	1.02± 0.11	1.12± 0.07
Tar-P	2.82± 0.25	2.18± 0.11	1.34± 0.10

*30 mg/kg of sample were treated to nine animals for three days.
Control group was injected vehicle only.

tivity.[24] The gas chromatogram showed that the polysaccharide was composed of five monosaccharides such as fructose as main component (42.94%), galactose, xylose, glucose and mannose. The amino acid composition of protein revealed four main acids (aspartic acid, cysteine, serine and glycine) and several minor acids (threonine, valine, glutamic acid, etc.). The streching frequencies of O-H (3,300-3,400 cm^{-1}) and C-H(2,900 and 1,600 cm^{-1}) usually means the characteristics of polysaccharide with the bending frequencies of C-H and O-H near, 1,000-1,100 cm^{-1}. A weak primary O-H bond of sugar containing sulfide group also appeared near 820 cm^{-1}.

Antitumor activities

The results of antitumor test with the sample exposed different phases in life span of ascites tumor-bearing mice and growth of solid type tumor. Lower used levels of the sample as 5 and 10 mg/kg showed weak or insignificant activity but higher level as 30 mg/kg showed significant inhibitory action on solid tumor growth. On the other hand, the same higher dose level showed insignificant prolongation effect of life span compared with control group (Table I). Viabilities of tumor cells cultured in proper medium showed no differences compared with those cultured in the same kinds of medium added with sample. Such results clearly imply that the polysaccharide have little or no direct cytotoxic activity against sarcoma-180 tumor cells.

Table III. Effect of Tar-P on the immunoorgan weight change of male ICR mice

Checked part	Weight in grams		% Increased
	Control	Treated	
Body wt.			
(1st day)	17.27± 0.30	16.45± 0.20	
(17th day)	27.08± 0.48	29.92± 0.68	
Organ wt.			
(17th day)			
Liver	1.63± 0.08	2.02± 0.08	23.93*
Spleen	0.21± 0.01	0.28± 0.01	33.33**
Thymus	0.07± 0.01	0.09± 0.01	28.57**

Data are mean value with standard error from nine animals.
Significance: *$p<0.05$, **$p<0.01$

Table IV. Effect of Tar-P on the Arthus reaction (antibody mediated hypersensitivity, AMH) and delayed-type hypersensitivity (DTH) in tumor bearing male ICR mice

Treatments*	Increase in footpad thickness (10^{-1} mm)	
	AMH	DTH
Normal mice	8.62	6.62
S-180 bearing mice	4.84	2.34
Tar-P treated S-180 Bearing mice	7.32	5.02

*Data are mean value from six animals which were treated with 30 mg/kg of sample for 10 days. Control groups were injected vehicle only.

Effects on immune function

In order to study more detailed mechanisms of antitumor activity, effects of the polysaccharide on total peritoneal exudate cells, immunoorgan weight, Arthus reaction and delayed-type hypersensitivity were tested. As shown in Table II, the sample trea-

ted group manifested an increase in number of peritoneal exudate cell according to sample administration before the 4th day but no significant increase thereafter.

Tar-P treated animals showed no significantly different body weight compared with control group. But the weights of immune function relating organs, such as liver, spleen and thymus were increased significantly (Table III). These phenomena may imply the increase of macrophage contained in the liver and spleen due to the sample and also the enlargement of the thymus according to the increase of T-lymphocyte activity.

The effects of Tar-P on the Arthus reaction and delayed-type hypersensitivity in tumor bearing mice are shown in Table IV. As shown in the Table IV, the activities were significantly depressed in sarcoma-180 tumor bearing mice, but were restored in the sample treated group. Such results may imply the activation of complements in antigen-antibody reaction or increase of T-lymphocyte reactivity in macrophage-antigen conjugation due to the action of Tar-P. But the amount total serum protein was not affected by Tar-P treatment (mean value 5.82g protein/d*l* serum in control group and 6.03 g/d*l* in Tar-P treated group, 103.6% of control, non-significant).

We have reported earlier that the polysaccharide fraction from the root of *Trichosanthes kirilowii* (Cucurbitaceae) showed potent antitumor activity and the activity may be caused not only by direct cytotoxicity but also by immunopotentiating effect.[25] In this study, results by the polysaccharide Tar-P treatments such as strong antitumor activity, the increase in the peritoneal exudate cell and immunoorgan weight can be explained as the manifestation of immune response related with thymus-dependent T-lymphocytes.

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