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Antitumor Activity of Some Phytobased Polysaccharides and their Effects on the Immune Function

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Abstract □ Polysaccharide fractions were prepared from Ginseng root, *Mori Radicis Cortex* (M.R.C.), *Phellodendri Cortex* (Ph.C.), *Sappan Wood* (S.W.) and *Tigli Semen* (T.S.). Water extract was also prepared from the mixture of Ph.C., S.W. and T.S. Ginseng polysaccharide and water extract of the mixture showed marked antitumor activity against sarcoma 180. Ginseng polysaccharide showed a mild increasing effect on the number of circulating leucocytes and a marked increasing effect on the number of plaque forming cells (PFC). Polysaccharides from ginseng root, S.W., Ph.C.+T.S. and water extract of the mixture showed dramatic inducing activities of peritoneal exudate cells (PEC), polymorphonuclear leucocytes (PMN) and macrophages. These results suggest the possibility that water extract of the mixture may have the lentinan like effect and ginseng polysaccharide may have stimulating effects on the general immune system.

Keywords □ Polysaccharides, Ginseng root, *Mori Radicis Cortex*, *Phellodendri Cortex*, *Sappan Wood*, *Tigli Semen*, Antitumor activity against sarcoma 180, Immunostimulating activity.

As the life pattern of man kind becomes more civilized and sophisticated, cancer hazard of the myriad chemicals to which our environments expose all of us, remains one of our most pressing health problems. Furthemore conventional treatment programs for tumor patients, such as surgical method, radiation therapy and chemotherapy are faced with some limitations in effects. Therefore, it is desirable to develope new nontoxic anticancer agent or cancer prophylactic agent from natural products[1].

It is well known that naturally occurring immunoadjuvants such as Bacillus-Calmette-Guerine (BCG)[2], lentinan from *Lentinus edodes*[3] and schizophyllan from *Schizophyllum commune*[4] manifest the antitnmor effect through the augmenting action on host immunological defense mechanisms[4-6]. Most of antitumor polysaccharides are practically nontoxic[4], which could be thought to be ideal cancer therapeutic agents. From ancient period, a number of medicinal herbs have been used for the treatment of malignant tumor in orient[7]. And some of them has an immune stimulating activity and it suggests that these provide the rational basis for their therapeutic uses as biological response modifiers[8].

In these points of view, we start to study the bioresponses of polysaccharides from plants. In the present study, some polysaccharides were examined for their antitumor activity and immune responses. For the evaluation of antitumor activities of polysaccharides, we have undertaken survival test and solid tumor growth inhibition test against sarcoma 180 and we also investigated the effects of polysaccharides on the immune responses, such as the number of

circulating leucocytes, lymphoid organ weight, the number of plaque forming cells and peritoneal exudate cell population.

EXPERIMENTAL METHODS

Materials

Ginseng root, T.S., Ph.C., M.R.C. and S.W. were commercially obtained from market. Male ICR mice were obtained from the Experimental Animal Farm of Seoul National University.

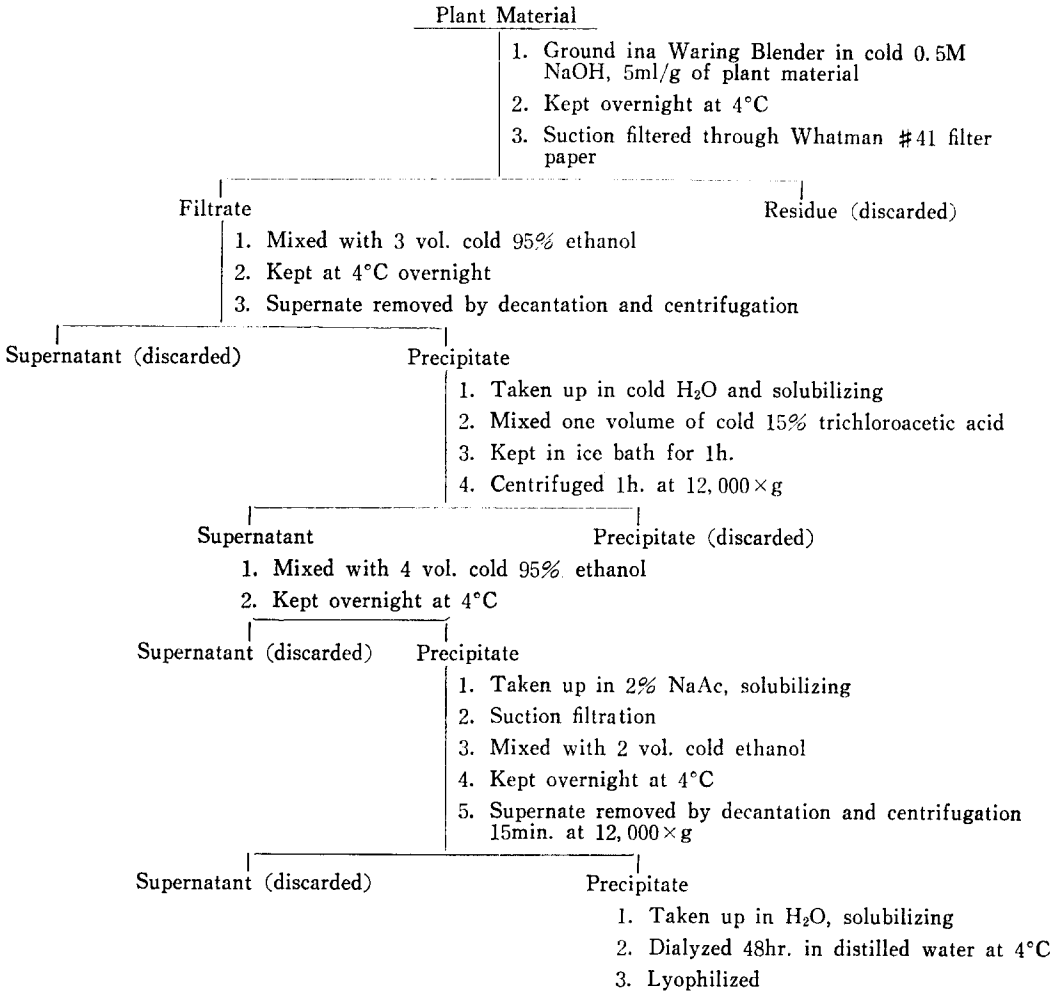
Sarcoma 180 was maintained by serial *i.p.* passage in ICR mice.

Preparation of Polysaccharides

Polysaccharides were extracted from the plant materials according to the scheme outlined in Scheme I[9]. Extraction procedure consisted of alkali extraction, serial ethanol precipitation, deproteinization, dialysis and lyophillization. Also, water soluble fraction was extracted from the mixture of Ph.C., S.W. and T.S.

Antitumor Test

1) Survival test: The male ICR mice received *i.p.* 0.1ml (1×10^6 cells) of ascites tumor from



Scheme I: Flow sheet for extraction procedure of water soluble polysaccharide from plants.

7-day-old sarcoma 180. Each dose of samples was injected *i.p.* once a day from 24 hours following transplantation and survival times of the mice were recorded. The survival period was compared with that of the control mice. The treated and control mice were recorded. The survival period was compared with that of the control mice. The treated and control groups each comprised 10 mice.

2) Solid tumor growth inhibition test: Antitumor test was carried out by approximately the same method as that reported for the antitumor activity of lentinan[3]. Male ICR mice weighing about 20g were subcutaneoulsy implanted with 1×10^6 cells of sarcoma 180 into the left groin 24 hours before the start of sample administration. Samples were administered once a day for ten days by *i.p.* injection with each dose. Twenty-one days after the tumor implantation, the mice were sacrificed and the solid tumors were excised and inhibition ratios were calculated from their weights.

Number of Circulating Leucocytes

The effects of polysaccharides on the number of circulating leucocytes were examined by following method[5]. Each group of ICR mice, 12 mice to a group, were given intraperitoneal injections of the polysaccharides for ten days. Blood was collected from three mice of each group from the retro-orbital venous plexus 2, 4 and 7 days after the sample injection. The blood was diluted with citrate saline, stained by Turk's reagent and the total number of leucocyte was counted. This number was compared with that obtained from mice.

Lymphoid Organ Weight

Male ICR mice weighing about 20g were injected with samples for ten days. Each group consisted of 5 male ICR mice. 7 days after the last injection, spleens were removed, weighed and compared with the result obtained from control mice.

Plaque Forming Cells

In order to examine whether these polysaccharides accelerated the antibody production to heterologous antigen or not, the slide technique of Cunningham[10] was used. Each dose of samples was injected *i.p.* once a day for 6 days into four groups of five mice. Four and seven days after the first sample injection, 0.1ml of 25% sheeep erythrocytes in saline was injected *i.p.*. Four days after the second immunization, the spleens were removed from the mice and the number of plaque formed by the antibody-forming cells from 1×10^6 spleen cells was counted by the conventional method.

Peritoneal Cell Population

Cell preparation was carried out by the following method. Briefly, samples were injected into the peritoneal cavity of male ICR mice and the peritoneal exudate was harvested 24, 48, 72 and 96 hours after the sample injection using phosphate buffered balanced salt solution (BSS). Exudate containing erythrocyte was hemolyzed with 0.83% ammonium chloride solution. Total cells were counted in hemocytometer chamber after cytocentrifugation (600 g, 10min.) and resuspending with 2ml BSS. After further centrifugation (600 g, 10min.), residues were resuspended with 1ml BSS and with the use of Giemsa and nonspecific esterase(NSE) staining[11], the number of PMN and macrophage were counted.

RESULTS AND DISCUSSION

The antitumor activity of polysaccharides was recognized by the suppression of the tumor growth and the prolongation of the life span of the sarcoma 180 bearing mice. As shown in

Table I: Effects of polysaccharides on survival of ICR mice transplanted *i.p.* with sarcoma 180 ascites.

Samples	Dose (mg/kg×days)	Number of mice	Number of tumor cells	Average survival days
Control	—	10	1×10^6	10.6
Ginseng	2×10	11	1×10^6	12.2
	20×10	14	1×10^6	13.6
Ph.C.+T.S.	4×10	11	1×10^6	15.5
Ph.C.	10×10	10	1×10^6	11.1
S.W.	10×10	10	1×10^6	10.6
Control	—	8	1×10^6	14.5
M.R.C.	2×10	10	1×10^6	12.7
	10×10	10	1×10^6	14.5
	20×10	10	1×10^6	11.4

Ph.C: *Phellodendri Corex* T.S.: *Tigli Semen*
S.W.: *Sappan Wood* M.R.C.: *Mori Radicis Cortex*

Table I, we found the prolongations of the life span in polysaccharides from Ginseng and Ph.C.+T.S. treated mice. These results are above the level NCI commented[12]. In tumor growth inhibition test, as shown in Table II, water extract of the mixture and Ginseng polysaccharide showed marked inhibition effects, but polysaccharides from Ph.C.+T.S. and S.W. did somewhat low inhibition effects. Complete regression was shown only for Ginseng polysaccharide at dose 20mg/kg, 2/9.

The effects of polysaccharides on the number of circulating leucocytes in mice is shown in Table III. The number of leucocyte in $1mm^3$ of blood drawn 2, 4 and 7 days after the administration of the polysaccharides was not significa-

Table II: Antitumor activities of polysaccharides against sarcoma-180 implanted *s.c.* into ICR mice.

Sample	N	Dose (mg/kg×days)	Body weight change (g)	Tumor weight (mean g±SE)	Inhibition ratio (%)	Complete regression
Control	10	—	+8.75	4.46±2.79	—	—
M.R.C.	10	2×10	+7.87	4.38±2.78	8	—
	10	10×10	+7.90	4.67±2.63	—	—
	10	20×10	+9.56	4.11±2.44	7.8	—
Control	10	—	+10.51	2.52±0.73	—	—
Mixture	9	0.1×10	+6.09	0.38±0.21	85	—
Ginseng	9	20×10	+5.17	0.65±0.60	74	2/9
	9	2×10	+6.58	0.79±0.32	69	—
S.W.	9	10×10	+9.49	1.01±0.47	60	—
T.S.+Ph.C.	9	4×10	+10.7	1.26±0.17	51	—
Ph.C.	9	10×10	+10.3	2.47±0.72	2	—

M.R.C,: *Mori Radicis Cortex* S.W.: *Sappan Wood* T.S.: *Tigli Semen*
Ph.C.: *Phellodendri Cortex* Mixture: mixture of T.S., S.W. and Ph.C.

Table III: Effects of polysaccharides on the number of circulating leucocytes.

(mean±SE/mm³)

Sample	N	Dose(mg/kg×days)	2nd day	4th day	7th day
Control	12	—	8600±1200	7600±200	6000± 500
Ginseng	12	2×10	8800± 200	7600±200	7500± 600
	12	10×10	8900± 400	9700±700	9100± 300
	12	20×10	8600± 100	9000±900	8200± 900
M.R.C.	12	2×10	8100± 600	6500±100	6700± 100
	12	10×10	8100± 300	8400±300	8100± 800
	12	20×10	8000± 300	6800±400	7800±1200
Control	12	—	7600± 700	8200±600	6500± 700
T.S.+Ph.C.	12	4×10	6900± 300	7000±500	6700± 300
Ph.C.	12	10×10	5700± 200	7600±400	8500± 500
Mixture	12	0.1×10	6900± 600	7600±700	7300± 400
S.W.	12	10×10	7400± 600	8600±700	8100± 900

M.R.C.: *Mori Radicis Cortex* T.S.: *Tigli Semen* Ph.c.: *Phellodendri Cortex*
S.W.: *Sappan Wood* Mixture: mixture of T.S., S.W. and Ph.C.

Table IV: Effects of polysaccharides on the spleen weight.

Sample	N	Dose (mg/kg×days)	Body weight (mean g±SE)	Spleen weight (mean g±SE)	S/B ratio (%)
Control	5	—	28.13±1.24	0.23±0.062	0.87±0.23
M.R.C.	5	2×10	25.87±2.44	0.19±0.029	0.74±0.13
	5	10×10	27.25±2.24	0.26±0.042	0.80±0.07
	5	20×10	25.30±0.70	0.24±0.005	0.97±0.03
Ginseng	5	2×10	26.58±1.57	0.19±0.046	0.73±0.15
	5	10×10	27.53±1.86	0.22±0.025	0.80±0.08
	5	20×10	26.83±2.90	0.15±0.012	0.58±0.11
Control	5	—	31.25±1.40	0.25±0.052	0.80±0.16
S.W.	5	10×10	29.16±2.42	0.22±0.043	0.74±0.17
Ph.C.	5	10×10	27.93±3.09	0.20±0.040	0.70±0.11
Ph.C.+T.S.	5	4×10	27.50±2.26	0.23±0.022	0.85±0.06
Mixture	5	0.1×10	26.58±1.36	0.21±0.046	0.78±0.19

M.R.C.: *Mori Radicis Cortex* S.W.: *Sappan Wood* Ph.C.: *Phellodendri Cortex*
T.S.: *Tigli Semen* Mixture: mixture of T.S., S.W. and Ph.C.

ntly different from that of control mice except Ginseng polysaccharide. Ginseng polysaccharide showed increasing effect, especially, on 4th and 7th day.

Significant differences in spleen/body-weight ratio were not observed among the samples. (Table IV)

The plaque forming test was carried out to show whether polysaccharides would increase the number of the antibody producing cells in the spleen ahainst sgeep erythrocytes. As seen in Table V, the number of PFC in mouse

Table V: Effects of polysaccharides on the number of PFC.

Sample	N	Dose	Plaque forming cells per 1×10^5 spleen cells
Control	5	—	720±210
Ginseng	5	20mg/kg×6	1220±370
Ph.C.+T.S.	5	4mg/kg×6	770± 80
Mixture	5	0.1mg/kg×6	740±240

Ph.C.: *Phellodendri Cortex*, T.S.: *Tigli Semen*
Mixture: mixture of T.S., S.W. and Ph.C.

spleen was not significantly different from that in control mice except Ginseng polysaccharide at 4th day after the end of sample administration. Ginseng polysaccharide showed increasing-effect on the number of PFC.

M.R.C. did not show dramatic effect on the peritoneal cell composition, but others which had some degree of antitumor activity, showed marked changes in the peritoneal cell composition. Especially, water extract of the mixture has strong inducing effect of PMN and NSE positive cells. Ginseng polysaccharide showed the similar alteration pattern to that of water extract of the mixture, but its inducing effect was lowered compared with that of water extract of the mixture. The number of PMN was highest at 24 hours after injection for all samples, although the number of macrophage reached to maximum at 48 hours. (Figs. 1-4)

As the results showed, some samples tested

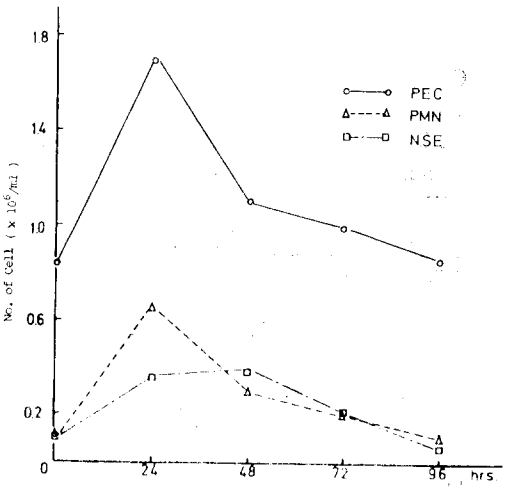


Fig. 2: Effect of polysaccharide from ginseng on the peritoneal cell population of ICR mice.

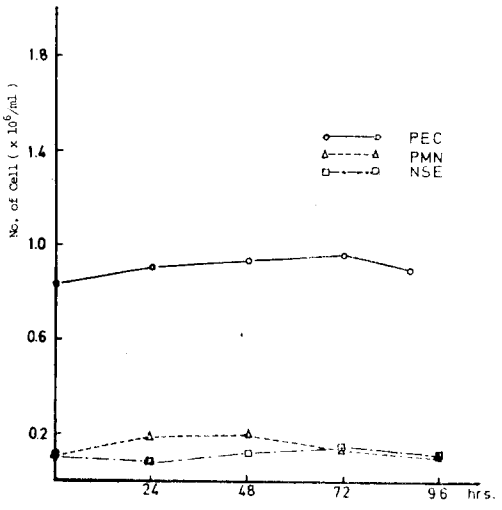


Fig. 1: Effect of polysaccharide from *Mori Radicis Cortex* on the peritoneal cell population of ICR mice.

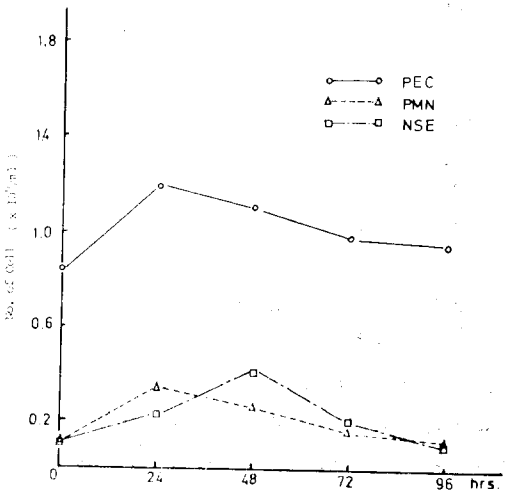


Fig. 3: Effect of polysaccharide from *Sappan Wood* on the peritoneal cell population of ICR mice.

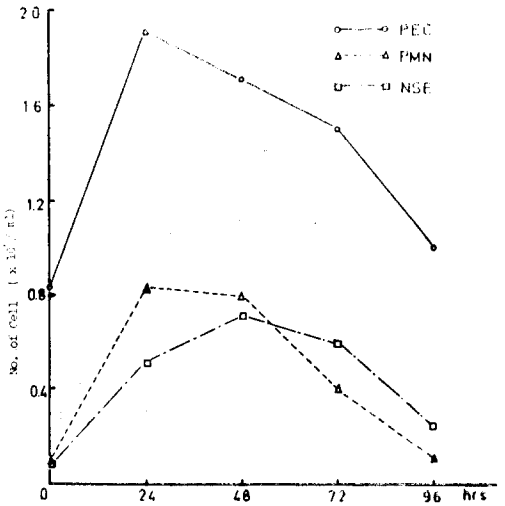


Fig. 4: Effect of water extract from mixture of Ph.C., S.W. and T.S. on the peritoneal cell population of ICR mice.

showed marked antitumor activities which are to be less than those of lentinan[3,6]. Taking into considering that these samples are crude, if more purifications are made, more strong effects could be expected. According to unpublished study, water extract of Ph.C. showed antitumor activity in vitro, but in this experiment, polysaccharide from Ph.C. was not proved for its antitumor activity. This fact suggests the possibility of the existence of other components of antitumor activity.

In the experiments on the effects on the immune responses, all the samples except Ginseng polysaccharide showed no modulating effects on general immune system. Water extract of the mixture showed marked effects on inducing PEC, PMN and macrophages. These effects of water extract of the mixture are somewhat similar to those of lentinan. That is, no effects on the general immune system, but significant effects on the tumor related immune system were observed[4-6].

Recently, a number of articles on the role of PMN against tumor cells have been reported[13-15]. PMNs play an important role in host defense against infection and are also capable of killing a variety of target cells including tumor cells[16,17] PMNs are cytotoxic to tumor cells in several in vitro model systems: during phagocytosis[18], in the presence of pharmacological reagents[19], in the presence of antibody directed against target cells and in the presence of lectins[15]. Although the mechanism of target cell killing are not fully understood, it appears that reactive oxygen species produced by PMNs play an important role in the lytic process[14]. Also, it is well known that macrophage has an important role in antitumor activity[20-25]. In this experiment, it was found that the stronger antitumor effect was proved, the more induction effect on macrophages and PMN was identified. The greatest number of macrophages was found at 48th hours after injection and PMNs reached to maximum at 24th hours. These results indicate the possibility that these preparations might have stimulating effect on proliferation from monocyte stem cell to macrophage.

The mechanism of action for water extract of the mixture is not yet determined, but according to an unpublished study (Moon, *et. al.*) and results obtained in this study, there might exist a combination of direct cytocidal effect and host mediated effect.

The effects of ginseng polysaccharide on tumor cells may be responsible for the stimulating effects on the general immune system. To elucidate the mechanism of action of these samples and to identify the true compon ents responsible for action, further studies are being continued.

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