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Studies on the Constituents of Higher Fungi of Korea(XXIII)

Antineoplastic Activities of *Coriolus versicolor* (L. ex Fr.)Quél., *Pleurotus ostreatus* (Fr.)Kummer and *Lentinus edodes* (Berk.)Sing.

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Abstract □ The carpophores of three Korean mushrooms, *Coriolus versicolor, Pleurotus ostreatus* and *Lentinus edodes* were respectively extracted with hot water and the extracts were dialyzed through Visking tube. They were found to exert an antitumor activity against sarcoma-180 implanted in mice. Especially, the inhibition ratio of the extract of *Coriolus versicolor* (100 mg/Kg, i.p.) was almost 100 %. But all the extracts did not affect the growth of leukemia L5187Y cells *in vitro*. Therefore these facts indicate that the extracts appear to stimulate cell-mediated immunity.

Keywords□ Basidiomycetes—the family *Polyporaceae*—*Coriolus versicolor, Pleurotus ostreatus,* and *Lentinus edodes.* Antineoplastic components—a protein-bound polysaccharide. Antitumor activity against sarcoma-180 implanted in mice.

Although more than 600 species of higher fungi were found in Korea[1], there have been not many reports of investigation on these fungi. Taxonomical and chemical studies on Korean higher fungi have been conducted by us for the past decade[1~10]. Though effects of the fungal extracts on microorganisms[11] and HeLa cell growth[12] were reported previously, pharmacologically active components of Korean higher fungi have been scarcely studied. Recently antineoplastic components of Japanese mushrooms have been extensively investigated[13~25]. Since antineoplastic activity of Korean fungi has not been studied, attempts were made to investigate antitumor activities of three mushrooms, *Coriolus versicolor, Pleurotus ostreatus* and *Lentinus edodes* which are readily available domestically. The results of these experiments showed that these mushrooms contained antitumor macromolecules.

EXPERIMENTAL

Materials

The carpophores of *Coriolus versicolor* (L. ex Fr.) Quél.(=*Polystictus versicolor* Fr.) which belongs to the family *Polyporaceae* were collected in Yangju Kun, (the Gwangneung area) Gyongki Do. The artificially cultivated carpophores of *Pleurotus ostreatus* (Fr.)Kummer and *Lentinus edodes* (Berk.) Sing. were used in the experiments.

Extraction and Isolation

The dried carpophores (200g) of *Coriolus versicolor* (L. ex Fr.). Quél.were homogenized

in a blender for five minutes and extracted with 0.1N NaOH by refluxing for seven hours. After filtration the residue was extracted with1. 51 of 0.1N-NaOH by refluxing for three hours. Then it was filtered and extracted again with0.8 1 of 0.4N-NaOH for two hours. The three filtrates were combined and evaporated in vacuum to 700 ml. It was dialyzed with Visking tube (Visking Co.) at 5°C for 72 hours until the colored low molecular weight material disappeared. It was then centrifugated with 5,000 rpm at 5°C for 10 minutes and the precipitate was removed. The supernatant was lyophillized in a freezing dryer (Edman Co.) and 30.03 g of a tasteless and odorless black-brown powder were obtained (15% yield), which was designated No. 419. The dried carpophores (200 g) of *Pleurotus ostreatus* (Fr.)Kummer were homogenized in a blender and extracted with three liters of water by refluxing for eight hours. After filtration, the residue was extracted with 1.5 1 of water by refluxing for four hours and then filtered. After the repeated extraction with one liter of water for four hours, the three filtrates were combined and evaporated to 700 ml in vacuum. It was dialyzed with Visking tube at 5°C for 72 hours until the colored low molecular weight material disappeared. The precipitate was removed by centrifugation at 5°C and the supernatant was lyophillized in a freezing dryer to obtain 9.36 g (4.82 %) of a tasteless and odorless light brown powder, which was here designated No. 405. The dried carpophores (200 g) of *Lentinus edodes* (Berk.) Sing. were homogenized in a blender for five minutes and extracted with two liters of water

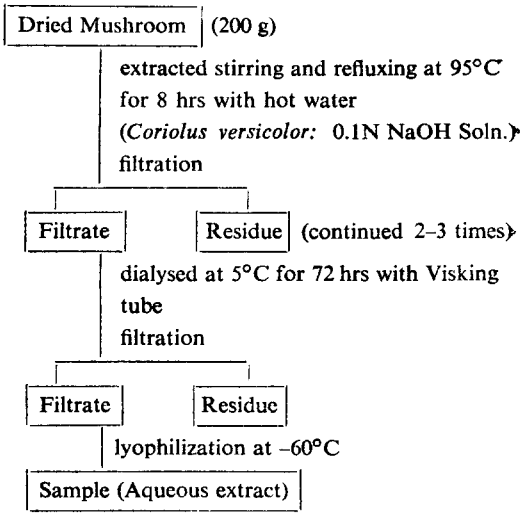

Fig. 1: Extraction and separation of the components from *Coriolus versicolor* (L. ex Fr.)Quél., *Pleurotus ostreatus* (Fr.)Kummer, and *Lentinus edodes* (Berk.)Sing.

by refluxing for eight hours. After filtration, the residue was extracted two times and the filtrates were combined and evaporated to 500 ml in vacuum.

After dialysis for 72 hours at 5°C, the precipitate was removed by centrifugating at 5°C for 10 minutes. By lyophillization, 6.22 g of a tasteless and odorless brown powder (6.22%) were obtained, which was designated No. 412.

Antitumor Test in Mice

The cells of sarcoma-180 were intraperitoneally transplanted in five mice (A-strain). After one week, sarcoma-180 cells were obtained from the ascite of the transplanted mice and transplanted to 70 mice (2×10^6 cell/0.1 ml) at its right axilla to induce solid tumor. Seven groups of 10 mice were divided into two groups: a control group and six treatment groups. The latter groups

received intraperitoneally 10 and 100 mg/Kg of No.405: 10 and 100 mg/Kg of No. 412: 10 and 100 mg/Kg of No. 419. A saline solution was injected intraperitoneally to the control group. One week after transplantation, sarcoma-180 bearing mice were injected i.p. with the above dose of the powder every other day 11 times for 20 days. Five days after the last injection, mice were killed and the solid tumor was ectomized to be wieghed. From tumor weight, mean value of each group of mice was calculated. Inhibition ratio (I.R.) as index of anticancer activity was calculated as follows:

$$\text{I.R.} = \frac{Cw - Tw}{Cw} \times 100$$

where Cw was mean weight of the tumor of the control group and Tw was that of the tumor of the treatment groups.

Effects on Cancer Cell Culture

Leukemia L5178Y cell was cultured in a medium which contained Fisher's medium (Grand Island Biological Co.) and 10 % horse serum. This culture served as control. It was also cultured in a medium which contained a mixture of Fisher's medium, 10 % horse serum and the aqueous extracts of the mushrooms. After 24 hour cultivation, the number of leukemia L5178Y cell was counted by Coulter counter (Model A Serial 981, Coulter Electronics Co.). Two-tenth gram of the mushroom extracts was dissolved in five ml of water, respectively and the precipitate was removed by centrifugation at 1000 rpm for five minutes and the solution was sterilized at 120°C for 30 minutes in an autoclave.

To 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml of each 4 % solution was added water to make each volume 5.5 ml, respectively. Fisher's Medium contained 0.73 mg/ml, 1.45 mg/ml, 2.90 mg/ml, 5.80 mg/ml and 11.6 mg/ml of each mushroom extract. The control contained only Fisher's medium. All sample and control groups were incubated at 37°C for 24 hours and then cells were counted with Coulter counter. At zero time, the number of the cell was 9.27×10^4 and the doubling time of leukemia L5178Y cell was 12 hours.

RESULTS

Anticancer Activities

The results of the anticancer effect *in vivo* of the three extracts as shown in Table I demonstrated that the aqueous extracts of three mushrooms, *Coriolus versicolor*, *Pleurotus ostreatus*, and *Lentinus edodes*, had a high antineoplastic activity. Especially, the extract of *Coriolus versicolor* exhibited much higher activity against sarcoma-180 than the extracts of the other two. Fig. 2 represents the life span of the groups of mice injected intraperitoneally and that of the control group and Fig. 3 represents the life span of the group of mice injected intraperitoneally 100 mg/Kg of the each mushroom extract and that of the control group. These two figures showed that the life span of the treatment group was longer than that of the control group. In the case of *Coriolus versicolor* with doses of 10 mg/kg and 100 mg/Kg lived vividly for 32 days' experiment. The results of this experiment showed that the extract of *Coriolus versico-*

Table I: Effects of the aqueous extracts of mushrooms on mice bearing sarcoma-180

	Average body weight (g)	Average tumor weight (g)	Inhibition ratio (%)	100 % Regression
Control (10 mice/group) i.p. saline	23±2.24*	4.73±1.02		0/10
No. 405				
10 mg/kg (i.p.)	22.5±2.65	1.89±2.20	60.0	1/10
100 mg/kg(i.p.)	22.1±2.45	1.76±2.05	62.8	2/10
No. 412				
10 mg/kg (i.p.)	21.3±2.45	1.25±2.23	73.6	0/10
100 mg/kg(i.p.)	22.±2.65	0.47±0.34	90.1	2/10
No. 419				
10 mg/kg (i.p.)	19.9±3.16	0.30	93.7	9/10
100 mg/kg(i.p.)	23.3±3.16	0.00	100.0	10/10

Mice were inoculated subcutaneously with sarcoma-180 (2×10^5 cell/mouse). Administration of aqueous extract of each mushroom was initiated seven days after inoculation of sarcoma-180. Body and tumor weights were measured on the 32nd day after inoculation. *Values are mean±standard deviation(SD).

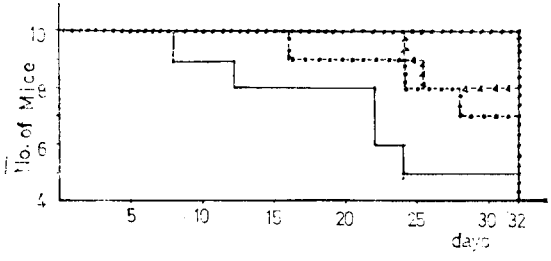

Fig. 2: Effects of the mushroom components (10 mg/kg/i.p.) on the life span of mice inoculated with sarcoma-180.
——control, —▽—▽—No. 405 (the extract of *P. o.*), —○—○—No. 412 (the extract of *L. e.*), —●—●—No. 419 (the extract of *C. v.*).

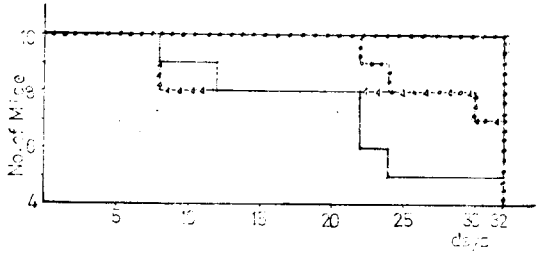

Fig. 3: Effects of the mushroom components (100 mg/kg, i.p.) on the life span of mice inoculated with sarcoma-180.
——control, —▽—▽—No. 405 (the extract of *P. o.*), —○—○—No. 412 (the extract of *L.e.*),
—●—●—No. 419 (the extract of *C. v.*).

lor had very high anticancer activity and very low toxicity.

Effects of the Mushroom Extracts on Cancer Cell Culture

The results of the tests on leukemia L5178Y cell culture *in vitro* as listed in Table II showed that the extracts of the three mushrooms affected the growth of the leukemia cell neither positively nor negatively.

DISCUSSION

The results of the above animal tests showed that the aqueous extracts of the three higher fungi had very high anticancer effect and especially in the case of *Coriolus versicolor*, almost 100% of inhibition ratio against mouse sarcoma-180 was obtained. There are

Table II: Effects of the mushroom components on the of leukemia cells *in vitro*

Sample	No. 405	No. 412	No. 419
Control	2.86×10^5	2.61×10^5	2.76×10^5
1* (0.73 mg/ ml)	2.94	3.11	2.78
2 (1.45 mg /ml)	2.96	2.83	2.75
3 (2.90 mg/ ml)	2.93	2.98	2.39
4 (5.8 mg / ml)	2.89	2.79	2.03
5 (11.6 mg/ml)	2.66	2.87	1.73

Zero time : 9.27×10^4 cell 24 hrs incubation.
* 1) 0.2 g of sample+5 ml of water
2) centrifuge
3) autoclave
4) 0.1, 0.2, 0.3, 0.4 and 0.5 ml/5.5 ml

numerous papers concerning the antitumor effect of polysaccharides isolated from various natural sources: for example, hemicellulose fraction from various plants[20,30] and polysaccharides from bamboo[31], fungi[32], lichen[33] and yeast[35,36]. Bacterial lipopolysaccharides[37] have been examined for their antitumor properties, mostly against mouse sarcoma-180. As one of immunotherapeutic agents, B.C.G. has been used for the treatment of melanoma, lung cancer, acute lymphatic leukemia and acute lymphoma.

It was already reported that these polysaccharides are capable of exerting immunostimulation in reactions that require the cooperation of thymus-dependent (T) and bone marrow-derived (B) lymphocytes[38]. Maeda *et al.*[19~22] reported that a polysaccharide, lentinan, from *Lentinus edodes* had about 90 % inhibition ratio against sarcoma-180 bearing mice, that it did not have anticancer effect against sarcoma-180 of thymus-ectomized mice[22], and that the effect of anti-lymphocyte serum (ALS) on the antitumor activity of lentinan was reduced[21]. They also stated that it stimulated the immune reaction of T lymphocytes. The results that the three mushroom extracts had the high antineoplastic effect against sarcoma-180 bearing mice in the same experimental procedure suggest that the aqueous extracts of *Pleurotus ostreatus* and *Coriolus versicolor* may also stimulate the cell-mediated immunity.

The results of the tests on cell growth *in vitro* in which they did not affect the growth of leukemia L5178Y cell suggest that the three mushroom extracts may stimulate immune response. These two experiments *in vivo* and *in vitro* indicate that their antineoplastic effect is not cytotoxicity but a stimulation of immune response. In the case of *Coriolus versicolor,* the mice which were injected with the aqueous extracts, neither died during the entire period of 32 days, nor showed reduction in their body weight. These facts suggest also that it may have a very low acute toxicity.

CONCLUSION

1) The aqueous extracts of *Coriolus versicolor, Pleurotus ostreatus,* and *Lentinus edodes* were found to exert the antineoplastic activity against sarcoma-180 implanted in mice and almost 100 % inhibition ratio especially in the case of *Coriolus versicolor* was observed.

2) The extracts did not affect the growth of leukemia L5178Y cell *in vitro,* either

positively or negatively. From the basis of the results on sarcoma-180 bearing mice and leukemia cell growth, the three mushroom extracts appear to stimulate cell-mediated immunity and to exhibit no acute cytotoxicity.

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