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Studies on the Toxicity of *Auricularia polytricha**

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털목이버섯(*Auricularia polytricha*)의 毒性에 관한 硏究*

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ABSTRACT

To screen biologically active components of the higher fungi of Korea, the carpophores of *Auricularia polytricha*, a well-known edible mushroom, were extracted with 0.14 M NaCl solution. The extractive was successively fractionated by adding ammonium sulfate in various amounts, the respective precipitates being separated by centrifugation, dialyzed and freeze-dried. When a dose of 60 mg/kg of each was, i.p., injected into ICR mice, the fraction which was precipitated at 20% $(NH_4)_2SO_4$ showed the highest toxicity, killing seven mice within two days. The fraction obtained at 40% $(NH_4)_2SO_4$ showed the second highest toxicity. The two fractions were named auratoxin I and II after the genus name. The symptoms of the intoxication were convulsion during the first 30 minutes after the injection, then sleeping within an hour, and tremor, lacrimation, nasal and ophthalmic bleeding, congestion and death in 24 hours. Particularly the spleen of the mice was found to be enlarged remarkably. The chemical analysis of the toxins showed that auratoxin I consisted of 4.4% protein and 84.5% polysaccharide and that auratoxin II 35.8% protein and 48.0% polysaccharide.

INTRODUCTION

The fungi of the genus *Auricularia* are well known edible mushrooms in the Orient, particularly in China and Korea. Two famous species are *A. auricula* and *A. polytricha*. Ukai and his associates in 1982 reported that an acidic heteroglycan was isolated from the carpophores of *Auricularia auricula* and that its molecular weight was about 300,000 to 370,000. In 1983 they found that this component had an antitumor activity. The reports on *Auricularia polytricha* began to appear in 1980. Hammerschmidt (1980) found that this mushroom contained an inhibitor of platelet aggregation. In 1981, Makheja and Bailey identified adenosine as the antiplatelet component. In 1982, however, Agarwal and his coworkers showed that another compound besides adenosine was responsible for the inhibitory activity. In 1981, Hokama[4] reported that the extracts of *Auricularia polytricha* and *Lentinus edodes* had a strong inhibitory compound of platelet aggregation. In 1983, Hokoma and his coworkers found a blastogenic inhibitory factor in the mushroom. In the course of our screening biologically active

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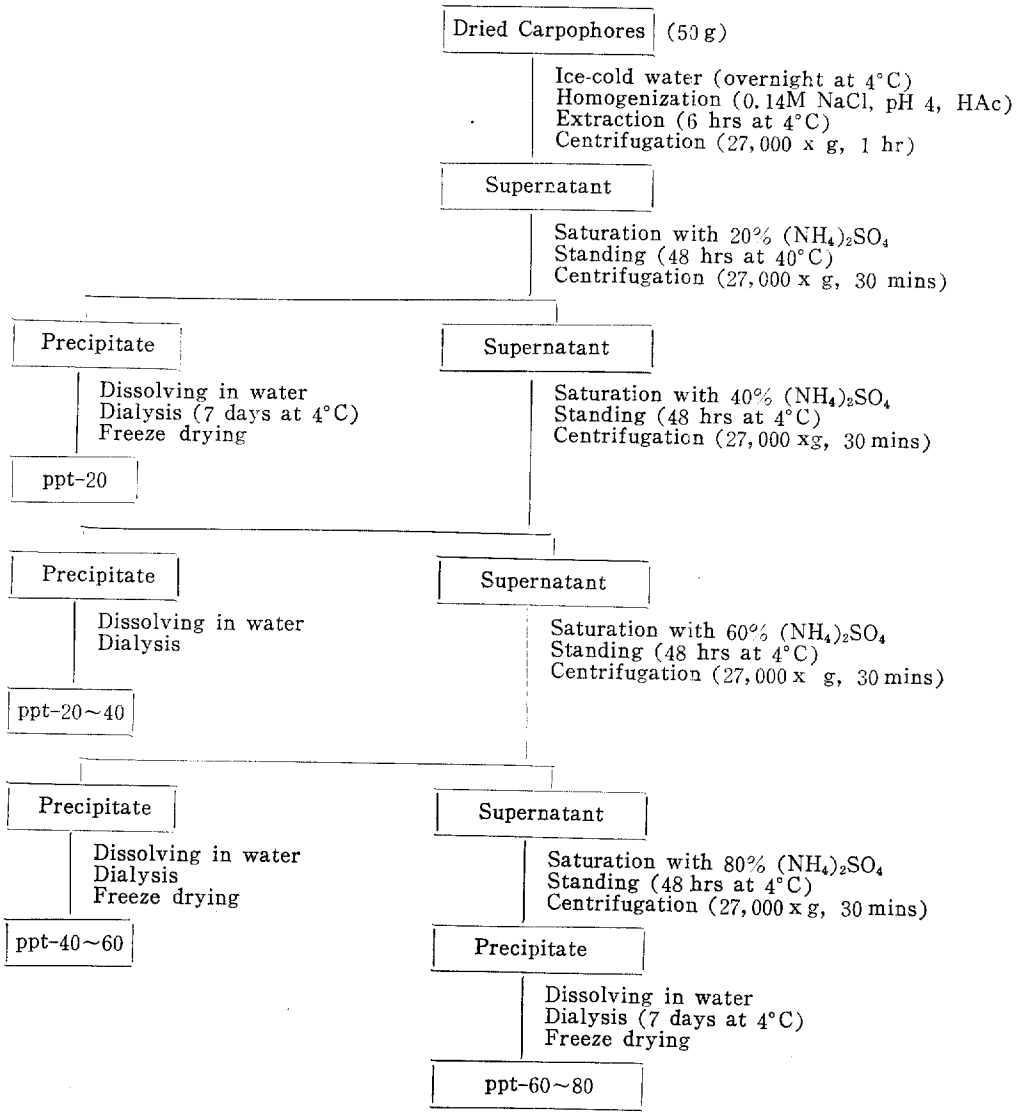

Fig. 1. Extraction and fractionation of the components from *Auricularia polytricha*(Mont.) Sacc..

components of Korean higher fungi, the acute toxicity of *Auricularia polytricha* was noticed in mice. However, no report of its acute toxicity has yet been found. This paper reports on the toxic components and their property of this edible mushroom.

EXPERIMENTAL METHODS

Materials

The dried carpophores of *Auricularia polytricha* (the family Auriculariaceae) used in this work were kindly provided by Agricultural Science Institute at Suwon, Gyeong-Gi Province, where they were cultivated.

Extraction and fractionation

The fruit bodies (50 g) of *Auricularia polytricha* were rehydrated in distilled water for 12 hours at 4°C and then homogenized and extracted with 0.14 M NaCl (pH 4) solution at 4°C for six hours. After centrifugation at 27,000×g for one hour the supernatant was saturated with 20% ammonium sulfate solution at 4°C. After 48 hours, the

precipitate was obtained by centrifugation at 27,000×g for 30 minutes, dissolved in distilled water, and freeze-dried after dialysis for seven days at 4°C. A dark brownish precipitate was obtained and designated as ppt-20. After the supernatant of ppt-20 was saturated 40%, 60% and 80% ammonium sulfate solutions successively and processed in the same method, ppt-20-40, ppt-40-60 and ppt-60-80 were respectively obtained (Figure 1). Freeze-dried ppt-20, ppt-20-40, ppt-40-60 and ppt-60-80 weredissolved in physiological saline for toxicity test.

Acute toxicity test

General acute toxicity and toxic manifestations were studied in female ICR mice. Mice weighing 18±2 g were randomly grouped with seven animals in each group. They were administered intraperitoneally at a dose of 60 mg/kg in an injection volume of 0.1 ml of the aqueous solutions of the precipitates and housed in individual cages provided with food and water. The toxic symptoms and behavioral changes exhibited by the animals were observed for 48 hours and dead mice were counted after the period of two weeks.

Effects on body and various organ weights

Changes in body and various organ weights were studied with 10 female ICR mice weighing 16.6~16.7 g. The aqueous solution of ppt-40 was intraperitoneally administered at a dose of 30 mg/kg in an injection volume of 0.05 ml singly and the control group was injected with saline. Their body weights were measured every day. After six days, each group was killed. The liver, spleen and kidney were removed, weighed and compared with those of the control group.

Chemical analysis

Total polysaccharide content: The quantitative analysis of the precipitate was conducted by using anthrone reagent and glucose was used as control. The color density was measured at 625 nm. By a calibration curve, the content of polysaccharide was calculated.

Total protein content: For the quantitative analysis of protein, samples were subjected to Lowry-Folin test and the absorbance of color was measured at 540 nm. By a calibration curve prepared by using bovine serum albumin as a standard protein, the protein content was determined.

RESULTS

Acute toxicity

The results of acute toxicity tests of ppt-20, ppt-20-40, ppt-40-60 and ppt-60-80 were shown in Figure 2. Of the four fractions tested, ppt-20 that was obtained by 20% ammonium sulfate solution showed the highest toxicity, killing all the seven mice within two days after single intraperitoneal injection. The second fraction, ppt-20-40 was the second highest toxicity, killing five of seven mice within three days. These toxic fractions, ppt-20 and ppt-20-40, were designated auratoxin I and II after the genus name of the mushroom.

Symptoms of intoxication

The typical symptoms of the intoxication were convulsion during the first 30 minutes after the injection, then sleeping within an hour, tremor, lacrimation, nasal and ophthal-

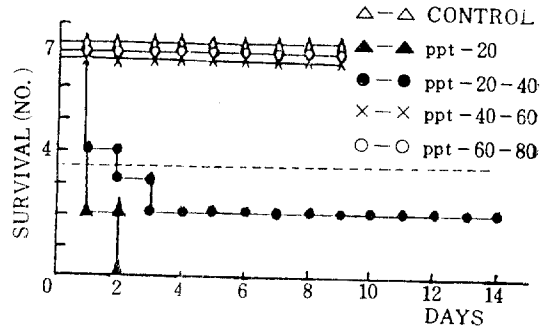

Fig. 2. Effect of acute toxicity of the extracts from *Auricularia polytricha* (Mont.) Sacc. on ICR female mice. A dose of 60 mg/kg of the extract in 0.1 ml was, i.p., injected.

Table Ⅰ. Clinical signs of Auratoxin Ⅰ and Ⅱ

Time	Clinical signs
20~30 min.	Convulsion
30~60 min.	Sleeping
24 hr.~	Tremor
	Lacrimation
	Bleeding(Eye, Nose, Urine)
	Congestion(Ear, Claw)
	Incapable of opening eyes
	Death

mic bleeding, congestion of ear and death in 24 hours. Whenever the injected mice were incapable of opening their eyes, these were most likely to die (Table Ⅰ).

Effects on body and organ weights

When a dose of 30 mg/kg of ppt-40 was intraperitoneally injected into female ICR mice, a mouse died after one day and the body weights of the remainders decreased rapidly and began to recover after one day (Figure 3). Six days after the injection of ppt-40, the average body weights of the injected group decreased to 90.1% when compared with those of the control group. The weights of spleen and liver increased, whereas the weight of kidney slightly decreased when compared with that of the control group(Table Ⅱ). However, when changes in percentage were based on the ratio of each organ weight to the body weight, those of the spleen, liver

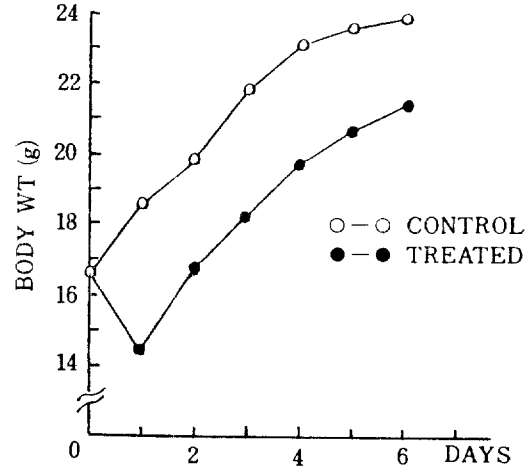

Fig. 3. Body weight change after injection of ppt-40 from *Auricularia polytricha*(Mont.) Sacc. A dose of 30 mg/kg of the extract in 0.05 ml was, i.p., injected to ICR mice. Each group was 10 mice and one mouse of treated group was dead after 1day.

Table Ⅱ. Effects of the ppt-40 of *Auricularia polytricha*(Mont.) Sacc. on the various organs of ICR female mice.

	Control(g)	Treated(g)	**Wt. Change(%)
Body Wt.(before injection)	16.62±0.27	16.70±0.32	—
Body Wt.(after 6 Days)	23.90±0.27	21.54±0.68 ($p<0.01$)	90.1
Spleen Wt.	0.16±0.01	0.27±0.02 ($p<0.01$)	168.8
Liver Wt.	1.59±0.04	1.73±0.09 (N.S.)	108.8
Kidney Wt.	0.45±0.01	0.41±0.02 (N.S.)	91.1
Spleen/Body Wt.($\times10^{-3}$)	26.70±0.45	12.32±0.82 ($p<0.001$)	183.0
Liver/Body Wt.($\times10^{-2}$)	6.66±0.19	8.07±0.41 ($p<0.01$)	121.2
Kidney/Body Wt.($\times10^{-2}$)	1.89±0.06	1.88±0.08 (N.S.)	99.5

Values were mean ± S. E.

** Wt. change = $\frac{\text{Treated Wt.}}{\text{Control Wt.}}$

N.S. = Not significant

One group was 10 mice.

and kidney were 183.0%, 121.2% and 99.5%, respectively.

Chemical analysis

Table Ⅲ shows the results of chemical analysis of auratoxin I and II obtained from 20% and 40% ammonium sulfate solutions. Auratoxin I consisted of 4.4% protein and 84.5% polysaccharide and auratoxin II 35.8% protein and 48.0% polysaccharide.

Table Ⅲ. Total protein and polysaccharide of Auratoxin I and Auratoxin Ⅱ.

	Total protein[a]	Total polysaccharide[b]
Auratoxin I	4.4%	84.5%
Auratoxin Ⅱ	35.8%	48.0%

a. Lowry-Folin Test
b. Anthrone Test

DISCUSSION

The results of these experiments demonstrate that the macromolecular components of *Auricularia polytricha*, one of the favorite edible mushrooms in the Far East, have a lethal toxicity when injected intraperitoneally into ICR mice. Since the mushroom has been consumed as a food without apparent toxic manifestations by humans for so many years, it was taken for granted as a safe food. However, only recently the reports on this mushroom began to appear, showing that it exerted an inhibitory activity against platelet aggregation and that the inhibitor may be adenosine or another compound of low molecular weight (Agarwal *et al.*, 1983; Hokama *et al.*, 1983; Hokama *et al.*, 1981; Makheja, 1981) These compounds may be removed during the period of the immersion of the mushroom in water which is an ordinary practice before actual cooking. Since the toxic components are protein-bound polysaccharides, most of them will remain within the mushroom. The first and second toxic fractions which were named auratoxin I and II by the authors were respectively composed of 4.4% protein and 84.5% polysaccharide and 35.8% protein and 48.0% polysaccharide. Although auratoxin I contained less protein than auratoxin II, the former was more toxic than the latter, indicating that the protein content did not directly correlate with the toxicity. Both toxins showed similar symptoms which were abdominal convulsion, tremor, lacrimation, nasal and ophthalmic bleeding, congestion of ear lobe and claw and coma. These symptoms were different from those of amatoxins and phallotoxins of the genus *Amanita*(Lincoff and Mitchel, 1977).

In the United States and some parts of Europe, physicians often encountered patients complaining of numbness at the back of the neck, headache, palpitation, lacrimation, and other unpleasant symptoms after their eating foods at Chinese restaurants (Kwok, 1968). These unusual symptoms are therefore called "Chinese restaurant syndrome."The physicians attributed it to monosodium glutamate that is abundantly used as an artificial meat flavor in Chinese foods, or to hypernatremia due to salt intake. However, we suggest that auratoxin I and II may be involved in causing the syndrome.

As far as we know, this is the first report on the acute toxicity of the protein-bound polysaccharide of the famous mushroom when it was injected into mice and can be at least a warning to the public for its unusual property. Further studies on chemical composition and toxic mechanism of auratoxins are in progress.

CONCLUSION

Two toxic components were isolated from the carpophores of *Auricularia polytricha*, an edible mushroom, and named auratoxin I and II. These toxins, when injected intraperito-

neally into mice, caused acute lethal intoxication. Auratoxin I contained 4.4% protein and 84.5% polysaccharide and auratoxin II 35.8% protein and 48.0% polysaccharide.

적 요

한국산 식용 버섯의 성분중 생리 활성물질을 검색하는 연구의 일환으로, 털목이버섯에서 추출한 고분자 물질을 마우스 복내강 주사하였을 때 강력한 급성 독성을 나타내었다. 털목이버섯을 0.14몰 염화나트륨 용액으로 추출한 후, 황산암모늄 20%, 40%, 60% 및 80%의 농도로 연속적으로 포화시켜 얻은 각 침전물을 원심분리, 투석 및 동결건조하였다. 이를 각각 ICR 마우스의 복강에 60mg/kg의 용량으로 주사한 결과 20% 및 40% 황산암모늄에서 얻은 분획이 강력한 독성을 발현하였으므로, 각각 auratoxin I 및 II 라 명명하였다. 그 급성 독성 증상은 주사후 20~30분 후부터 경련을 유발하였으며, 30~60분 후에는 모두 수면상태가 되었다. 24시간 후에는 전율, 코피, 눈물과잉분비 및 개안 불능등의 증상을 나타냈으며, 결국 사망하였다. 각종 장기무게 변화중 특히 비장의 무게가 증가하였다. 화학 분석 결과 auratoxin I은 단백질 4.4%와 다당류84.5%로 구성 되었으며, auratoxin II는 단백질 35.8%와 다당류 48.0%로 구성되어 있었다.

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