

Studies on Constituents of the Higher Fungi of Korea(XLVIII)*

Nematotoxin of *Naematoloma fasciculare*

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韓國產 高等 菌類의 成分 研究(第48報)

노란다발버섯의 毒 成分 네마톡신

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Abstract: To find toxic components in Korean higher fungi, the carpophores of *Naematoloma fasciculare* which had caused several cases of lethal intoxication were examined for toxicity. The components of high molecular weight were separated by ethanol precipitation and dialysis from the aqueous extract of the carpophores. After the components were freeze-dried, a brown powder was obtained. When a dose of 60mg/kg of this macromolecular fraction was intraperitoneally injected into mice, the mice began to die in six days and a half of them died within seven days. This toxic component was named nematotoxin after the genus name of the mushroom.

Keywords: *Naematoloma fasciculare*, Strophariaceae, Basidiomycetes, Toxin, Mycotoxin, Nematotoxin, Poisonous mushroom.

The carpophores of *Naematoloma fasciculare* which is one of the common wild mushrooms in Korea have frequently caused lethal intoxication (Kim, 1969; Tyler, 1963; Imazeki and Hongo, 1965; Lincoff and Mitchel, 1977). For instance, four persons were poisoned after ingestion of wild mushrooms in Pusan, July, 1968. The two members of a family in Incheon City died by mushroom poisoning in August, 1972. During the period of the subsequent month 18 people died and 23 were seriously ill by the intoxication of wild mushrooms in Gang-Won Province alone. One of these mushrooms was identified as *Naematoloma fasciculare* by the authors. Three sisters in a family

died after eating wild mushrooms in Uijungbu, Gyong-gi Province.

Two groups of investigators found, independently, two biologically active components in this mushroom. Ito *et al.* (1967) reported the isolation of naematolin from the fermentation broth of the mushroom. It showed cytotoxic activity against HeLa cells and inhibited the growth of polio virus and vesicular stomatitis virus. Furthermore it showed coronary vasodilating action to the isolated guinea-pig heart. Its LD₅₀ in mice was 400 mg/kg (*i.v.*).

Ikeda *et al.* (1977 and 1978) found plant growth inhibitors in this mushroom and named them fascic-

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ulols A, B and C. They determined the chemical structures of these three derivatives, and also isolated four analogs, namely, fasciculols D, E, F, and G. Lincoff and Mitchel (1977) described the mushroom as a poisonous one, even deadly, in certain locations. In nine hours after ingestion of the mushroom the onset of gastrointestinal symptoms may occur and extensive liver and kidney damage may develop. These are signs of toxic cyclopeptide poisoning.

Despite of such poisonous property (Lee *et al.*, 1959; Singer, 1975; Kim, 1978; Rayner, 1979; Pacioni and Lincoff, 1981; Moser, 1983), however, no report identifying the toxic component has yet appeared (Benedict, 1970; Benedict, 1972; Tyler, 1971; Hatfield, 1975; Turner and Aldridge, 1982). This fact led us to undertake this study and we report here the toxin of high molecular weight in this mushroom.

Materials and Methods

Materials

The fresh carpophores of *Naematoloma fasciculare* (Fr.) Karst. (the family Strophariaceae) were collected at the Bupyong Country Club in Bupyong, Gyeong-gi Province in September 10, 1983.

Methods

The carpophores were homogenized with distilled water in a blender and extracted by boiling on a water bath for eight hours. After the centrifugation of the extract at 8000 x g for half an hour, the supernatant was added with a three-fold volume of ethanol at -20°C to precipitate polymers of the components.

Wet carpophores, 20g

Homogenization
Extraction with hot water
Centrifugation(8000 x g, 30 min)

Supernatant

Ethanol(x 3, -20°C)
Centrifugation(8000 x g, 30 min)

Precipitate

Dissolving in water
Dialysis at 4°C, 4 days
Freeze-drying

Brownish powder

Fig. 1. Extraction of the macromolecule from *Naematoloma fasciculare*.

The precipitate was separated by centrifugation at 8000 x g for half an hour. Then it was dissolved in distilled water and dialyzed by Visking tube at 4°C for four days. After it was freeze-dried, a brownish powder was obtained and used for toxicity test (Fig. 1).

A dose of 60 mg/kg of the macromolecular fraction of the mushroom in a volume of 0.1 ml sterile distilled water was intraperitoneally injected into ICR female mice. Thereafter the mice were observed for toxicity.

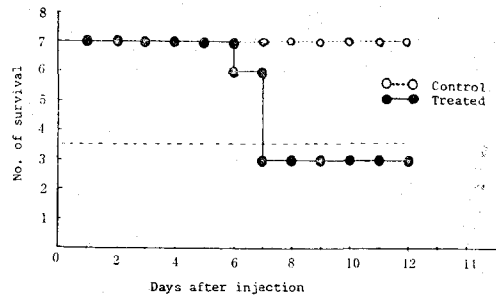


Fig. 2. The toxicity of *Naematoloma fasciculare* on ICR female mice (15±2g). A dose of 60 mg/kg of the macromolecule in 0.1 ml was, *i.p.*, injected.

Results and Discussion

When the macromolecular fraction of the carpophores of *Naematoloma fasciculare* was injected into ICR mice, they began to die in six days after the injection and four of seven mice died within seven days (Fig. 2). Since no apparent symptoms of acute toxicity appeared, it seems that the toxin may not be of cyclopeptide type. It appears to be less acute than that of cyclopeptide toxins. Rather the toxin may be a protein-bound polysaccharide which was isolated in the identical method from the carpophores of *Auricularia polytricha* by the authors and which showed lethal toxicity in ICR mice (Kim *et al.*, 1983). The chemical analysis of the toxic macromolecule of *Naematoloma fasciculare* is in progress.

Conclusion

The carpophore of *Naematoloma fasciculare* was

found to contain a macromolecular fraction which showed lethal toxicity when it was intraperitoneally injected into ICR mice. The toxic component was named nematoxin after its genus name.

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적 요

한국에 야생하는 독버섯의 하나인 노란다발버섯을 물로 가열 추출한 후 추출액에 3배의 에탄올을 첨가하여 고분자 물질을 침전시켰으며 이를 다시 투석하여 정제 하였다. 이것을 냉동 건조하여 분말을 얻었으며 60mg/kg 용량을 백서의 복강내에 주사 하였던 바, 6일 후 부터 사망하기 시작하여 7일째에는 7마리중 4마리가 절명하였다. 이 유독 성분을 "네마톡신"이라 명명하고자 한다.

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