

Study of Antiprotozoal Activity and Mitogenic Effects Within Some Medicinal Mushrooms

Suzanna M. Badalyan & S. H. Sisakyan

Laboratory of Fungal Biology and Biotechnology, Yerevan State University, Department of Botany, 1 Aleg Manoogian St., 375025, Yerevan, Armenia; M. Heratzi Yerevan State Medical University, Department of Medical Biology and Genetics, 2 Koryun St., 375025, Yerevan, Armenia

Many mushrooms possess both excellent nutritional values and pharmaceutical properties. Since ancient times, medicinal mushrooms have been used in traditional medicine of Asian countries. Isolated and identified substances, particularly from *Lentinus edodes* (Berk.) Singer, *Coprinus comatus* (O.F.Müll.) S.F.Gray, and *Flammulina velutipes* (W.Curt.:Fr.) Singer are used in the treatment of immune system disorders, cancer, bacterial, viral, and fungal infections, etc. They are able to decrease high glucose and lipid levels in the blood and are recommended as neuro- and vasotonics, hepatoprotective and thrombolytic agents. However, the antiprotozoal and mitogenic activities of mushrooms have not been sufficiently investigated yet.

Presented results, as a part of our Medicinal Mushrooms Myco-Pharmacological Screening Program (MMMPSP), concern the study of the antiprotozoal activity (APA) and mitogenic effects (MGE) of mycelium and fruiting bodies (FB) samples of *Lentinus edodes*, *Coprinus comatus*, and *Flammulina velutipes*.

Mycelia of tested species were cultivated in stationary conditions on 2% malt-extract liquid medium during 28 days. The cultural filtrate (CF) and mycelial extract (ME) samples, obtained on the 21st, 25th, and 28th days of growth, as well as FB extract of *F. velutipes* and separated polysaccharide-protein (PSP) fraction, were tested against unicellular protozoa *Paramecium caudatum* cultivated in microtiter plates using previously diluted (in water

in a 1:20 ratio) Lozinskii solution (NaCl-1 g, KCl-0.1 g, CaCl₂-0.1 g, MgSO₄-0.1 g, NaHCO₃-0.2 g in 0.5 L distillate water, pH 7.2) at 18–20°C. During the experiment, *P. caudatum* were fed with yeast extract.

Three different amounts (0.03, 0.06, and 0.09 mL) of five CF dilutions (not diluted and diluted in ratios 3:1, 2:1, 1:1, and 1:2) and four ME concentrations (0.01, 0.03, 0.05, and 0.1%) were tested on their APA and MGE. The APA was determined by the breakdown period of *P. caudatum* (in minutes or hours) and calculated in percents. The MGE was calculated in comparison with the mitotic division rate in control wells.

The CF and ME samples of investigated species revealed strong APA and significant MGE, respectively. A 0.09 mL CF (not diluted) of *Lentinus edodes* showed the highest APA (5 minutes), whereas previously diluted CF had relatively weaker activity (21–22 hours) (Table 1). The CF of *L. edodes* expressed no MGE. The ME (0.1 and 0.5%) possessed weaker APA compared to CF samples (48 hours). Lowering of the extract concentration brought the APA level decrease. No activity was found at 0.01% of ME. The low concentrations of ME (0.03, 0.01%, and 0.03 mL of 0.05%) possessed a 1.9–2.3-fold increase MGE.

All tested CF amounts of *Coprinus comatus* possessed strong APA. *Paramecium caudatum* were destroyed during 20 minutes. The level of activity was directly proportional to its tested amounts. No MGE

TABLE 1. Antiprotozoal Activity (APA) and Mitogenic Effect (MGE) of Medicinal Mushrooms *Lentinus edodes*, *Coprinus comatus*, and *Flammulina velutipes*

Species	Tested mushroom samples and their activities							
	CF		ME		FB extract		PSP fraction	
	APA	MGE	APA	MGE	APA	MGE	APA	MGE
<i>C. comatus</i>	20 min	0	0	×1.1–2.8	NT	NT	NT	NT
<i>F. velutipes</i>	48 hrs	×1.1–1.3	0	×1.2–2.2	0	×1.2–1.8	0	×1.1–1.4
<i>L. edodes</i>	22 hrs	0	48 hrs	×1.9–2.3	NT	NT	NT	NT

Notes: CF: cultural filtrate, ME: mycelial extract; FB: fruiting body, PSP: polysaccharide-protein; NT: not tested.

was observed within *C. comatus* CF. After 2 days of experiment, up to a 2.8 times increased MGE was detected within 0.1% ME samples of *C. comatus*, particularly, in the amount of 0.09 mL. Meanwhile, mitosis-stimulating activity was observed starting from 21 hours. A strong dose/effect correlation was revealed. At the same time, the tested ME concentrations of *C. comatus* did not possess any APA.

The CF samples of *Flammulina velutipes* showed weaker APA (48 hours) than tested CF samples of *Coprinus comatus* and *Lentinus edodes*. At the same time, a weak MGE was revealed when 0.03 mL of undiluted (×1.2), and diluted in ratio 2:1 (×1.3) and 1:2 (×1.1) CF of *F. velutipes* were used. All tested amounts of ME, particularly 0.06 and 0.09 mL of 0.05% and 0.1% during the 48 hours of observation, possessed up to a 2.2 times increase in MGE. It was almost absent at 0.01% of ME. No APA was observed within ME samples, FB extract, and PSP fraction separated from the extract of *F. velutipes*. However, they were able to stimulate mitosis of *Paramecium caudatum* on the

4th day of the experiment up to 2.2, 1.8, and 1.4 times, respectively.

No significant differences were found between APA and MGE levels of all tested CF and ME samples of three species obtained on the 21st, 25th, and 28th days of mycelial cultivation.

Thus, the presence of APA and MGE within *Lentinus edodes*, *Coprinus comatus*, and *Flammulina velutipes* completes the list of medicinal properties of these mushrooms and makes them suitable for further development of new mushroom-based nutritional supplements. They could be used in prevention and treatment of many intestinal protozoal infections and wound-healing processes.

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