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Pharmacological Activity of the Mushrooms *Flammulina velutipes* (Curt.: Fr.) Sing., *Paxillus involutus* (Batsch: Fr.) Fr., and *Tricholoma pardinum* Quél. (Basidiomycota)

Suzanna M. Badalian,[1] Jean-Jacques Serrano,[2] Sylvie Rapior,[2] and Claude Andary[2]

[1]Yerevan State University, Department of Botany 1, A. Manoogian St., 375025, Yerevan, Armenia, and [2]Faculty of Pharmacy, University of Montpellier 1. 15 Av. Ch. Flahault, 34060, Montpellier Cedex 2, France

ABSTRACT: Three mushroom species—*Flammulina velutipes, Paxillus involutus,* and *Tricholoma pardinum* (Basidiomycota)—were investigated for pharmacological activities on mice after intraperitoneal and *per os* administration of fruiting body extract. In particular, analgesic and spasmolytic activities of the mushroom extracts as well as their effects on the central nervous system were reported. Both methanol-soluble and water-soluble residues separated from methanol extracts of fruiting bodies of mushrooms were used. The mushroom extracts did not show any particular analgesic effects. However, extracts demonstrated algogen activity, especially *P. involutus.* Significant spasmolytic papaverine-like activity was also observed for *P. involutus,* as revealed by decreasing intestinal passability and diarrhea. Extracts of *P. involutus* and *T. pardinum* produced effects on the central nervous system of mice.

KEY WORDS: *Flammulina velutipes, Paxillus involutus, Tricholoma pardium,* analgesic and spasmolytic activities, mushroom extracts.

INTRODUCTION

The pharmacological activity of mushrooms has not received sufficient attention. In view of recent advancements in mycopharmacological studies, interest in mushrooms as a natural source of valuable bioactive metabolites with a large spectrum of pharmacological actions (antibacterial, antifungal, antiviral, antitumoral, hypoglycemic, fibrinolytic, neurotoxic, immunomodulating, antiinflammatory, and other activities) has significantly increased (De Bernardi et al., 1983; Fujimoto et al., 1986, 1991, 1992; Suzuki et al., 1989; Obuchi et al., 1990; Takazawa and Kashino, 1991; Kiho et al., 1992, 1994; Takahashi et al., 1992; Becker et al., 1994; Kobayashi et al., 1994; Zhang et al., 1994; Zhuang et al., 1994, 1996; Liu et al., 1996; Wang et al., 1996; Badalyan and Serrano, 1999).

Particular data concerning spasmolytic activity of mushrooms is rare. The papaverine-like relaxation effect was originally reported after intraperitoneal (i.p.) administration of three toxic metabolites separated from *Hebeloma spoliatum* (Fr.) P. Karst. at a dose of 45 mg/kg (Fujimoto et al., 1992). The similar administration at a dose of 100 mg/kg caused death after paralysis of the limbs in mice. Some other fungal triterpenes and sesquiterpenes (neurotoxins) also induce paralysis of legs and the nervous system in mice and sometimes can be lethal (Fujimoto et al., 1986, 1991; Badalyan et al., 1995). Therefore, the toxic

ABBREVIATIONS

i.p.: intraperitoneal method; **MSR:** methanol-soluble residues; **p.o.:** *per os*; **WSR:** water-soluble residues.

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mushroom compounds seemed to affect not only the central nervous system but also the autonomic nervous system in mice.

No other available data were found regarding analgesic or algogen activities of mushrooms.

This article examines pharmacological activities, algogen, analgesic, and spasmolytic, in particular, of three mushroom species: *Flammulina velutipes* (Curt.: Fr.) Sing. (edible), *Paxillus involutus* (Batsch: Fr.) Fr., and *Tricholoma pardinum* Quél. (poisonous). It is well known that *P. involutus* causes gastrointestinal and cardiovascular disorders, often with hemolytic effects (*Paxillus* syndrome). The *T. pardinum* can cause disturbances of the nervous system and gastrointestinal colic (Bresinsky and Besl, 1990). Data concerning pharmacological activities, such as antitumor and fibrinolytic activities, of edible *F. velutipes* can also be found (Watanabe et al., 1964; Hirano et al., 1987).

MATERIALS AND METHODS

Fungal Material

The fruiting bodies of *F. velutipes, P. involutus,* and *T. pardinum* were collected in Armenia, Germany, and France, respectively, and identified in fresh material.

Preparation of Extracts and Their Fractionation

Powdered fruiting bodies were extracted using methanol (5g, 100 ml) with magnetic agitation at room temperature as shown in Fig. 1.

After 2–3 weeks at 4°C, a crystalline precipitate of the concentrated extract was separated from the supernatant. The precipitate was washed with methanol and centrifuged. The supernatant was added to methanol–water (1:1) and freeze-dried. Water-soluble residues (WSR) and methanol-soluble residues (MSR) were added to 3% carboxymethylcellulose and used for the pharmacological studies.

Experimental Conditions

Adult EOPS Swiss mice, 5–6 weeks old, weighing 20–26 g, were used. The animals were housed three or five per cage (25 × 45 × 15 cm). Adaptation of the animals took place at room temperature (22°C) and relative humidity of 60 ± 10%. The mice were set on a 12-h light/dark cycle. They had free access to a commercial pellet diet (UAR AO4) and tap water *ad libitum.*

Suspensions of the residues (MSR, WSR) in 3% aqueous carboxymethylcellulose were administered intraperitoneally (i.p.) (10 ml/kg) and *per os* (p.o.) (20 ml/kg).

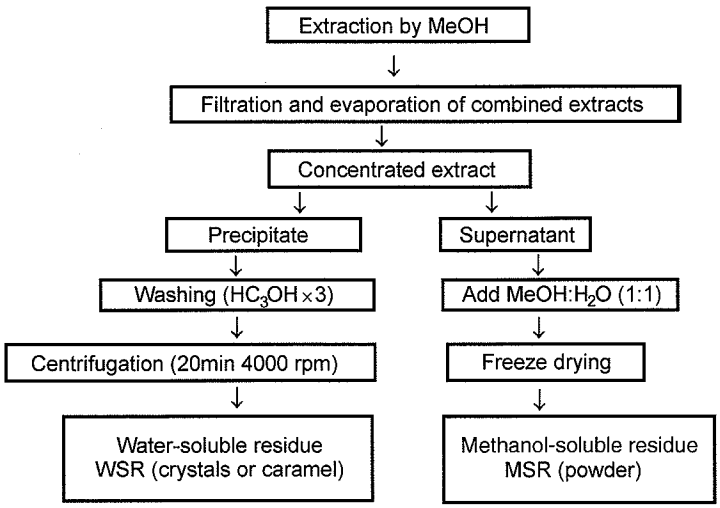

FIGURE 1. Extraction and purification of mushroom extracts.

Pharmacological Tests and Doses

Pharmacological Activity

To estimate pharmacological activity and toxicological evaluations of mushroom extracts, various pharmacological tests, such as the platform, traction, dynamic activity, catalepsy tests, as well as flexion, posture, pineal, corneal, and Haffner's reflexes were used. Tests were recorded twice in the first hour and every hour during the following 6 h, on days 5, 10, and 14. The animals were weighed at days 1, 5, 10, and 14.

Single i.p. and *per os* administration of both WSR and MSR were realized at different doses. For WSR the following doses were used: 1000–1500 mg/kg (*F. velutipes*), 500–750–1000 mg/kg *(P. involutus),* and 250–500–1000 mg/kg *(T. pardinum).* For MSR the following doses were used: 1000–1500 mg/kg (*F. velutipes*), 500–750–1200 mg/kg (*P. involutus,*) and 500–1000–2000 mg/kg *(T. pardinum).* Each dose was tested on three mice. Calculations of the pharmacological results were done in four possible ways: 0 = 0%; 1/3 = 33%; 2/3 = 66%, and 3/3 = 100%.

Analgesic Activity

Estimation of analgesic activity of mushroom extracts *in vivo* was performed on mice in the same experimental condition using Coster's method. According to this method, 1% acetic acid (0.2 ml/20 g) was injected intraperitoneally as an algogen factor provoking pain and contractions of dorsoabdominal muscles. To examine whether injected (i.p.) mushroom extracts might have analgesic or algogen effects, the following doses of MSR and WSR were used: *F. velutipes* and *P. involutus,* 1000 mg/kg each and *T. pardinum,* 800 mg/kg each. Analgesic or algogen activity was calculated by numbers of colic cramps using the following formula: $P\% = 100 \times (K - C)/K$, where K is the number of cramps in control animals; C is the number of cramps in animals after administration of mushroom extracts.

Effect on the Central Nervous System

The extracts from the three species were investigated using Buasie's test, which allows the study of dynamic activity and curiosity of the animals for 5 min at 45 min after *per os* administration of mushroom extracts (20 ml/kg). In this experiment the following doses of residues were used: WSR from *F. velutipes* and *P. involutus*—1000 mg/kg each, from *T. pardinum*—1200 mg/kg; MSR from *F. velutipes*—900 mg/kg, *P. involutus*—1200 mg/kg, and *T. pardinum*—350 mg/kg. Each dose was tested on five mice.

Spasmolytic Activity

Mushroom extracts were examined for spasmolytic activity after *per os* administration of a carbon suspension (10 mg/ml), which allows observation of the movement of intestinal contents. In this experiment three doses of residues from two species were included: *F. velutipes,* 1000 mg/kg (WSR) and 1200 mg/kg (MSR) and *P. involutus,* 350 mg/kg (MSR). For each dose three mice were used.

Alteration of intestinal passability ($D\%$) was calculated by using the following formula: $D(\%) = c(k) - c/c(k) \times 100$, where $c = a/b \times 100$; a is the length of intestinal passability; b is the total length of intestine; $c(k)$ is the passability within control mice; $c(k) - c$ is the diminution of passability; and D is passability in percent.

All results were statistically evaluated using Student's t-test (Oivin, 1960).

RESULTS AND DISCUSSION

Estimation of Pharmacological Activity

No deaths or significant pathological changes were seen in the animals after single intraperitoneal or *per os* administration of mushroom residues at doses ranging from 350 to 2000 mg/kg. These results were confirmed by animal weights at different doses for the same period of time (unpublished data). Autopsy of animals killed on day 14 did not reveal digestive, pulmonary, or other significant changes. Also, no significant changes of Hafner's reflex were observed. However, extracts from *P. involutus* and *T. pardinum* caused piloerection, increasing or decreasing dynamic activity in 66% of mice, as well as diarrhea. These

data were confirmed by complementary pharmacological tests, such as curiosity (Buasie's test) and spasmolytic effect (intestinal passability) of tested residues.

It was reported that pharmacological problems provoked by *per os* administration of mushroom extracts were more significant than problems due to i.p. administration. At the same time, it was mentioned that the WSR tested from mushroom extracts were more active pharmacologically than MSR. Previous chemical analyses of these mushroom residues have shown that the WSR contain more sugars, polysaccharides, polyols, and terpenoids, whereas the MSR mostly contain alkaloids, amino acids, ninhydrin-positive and indolic compounds, as well as phenolic acids (Badalyan, 1998).

Analgesic Activity

Tested extracts from fruiting bodies of *F. velutipes, P. involutus,* and *T. pardinum* did not show stable analgesic effects. However, during the first 5 min of the experiment, reinforcement and augmentation in the number of cramps were observed, particularly after using the residues (WSR, MSR) from the *P. involutus* extract. Statistical data ($p < 0.05$) show an average number of colic cramps during the 20 min of the experiment compared to control data. After a few minutes the situation stabilized (Table 1).

The total number of colic cramps calculated by the aforementioned formula in comparison with control for both WSR and MSR were: 4.13% and 6.61% (*F. velutipes*), 16.52% and 24.79% (*P. involutus*), 0.20% and 10.74% (*T. pardinum*), respectively. The results showed that intraperitoneal administration of MSR produced more colic cramps in animals in comparison with control.

Effect on Central Nervous System

Per os administration of all tested mushroom extracts for 5 min increased animal activity and responses. The WSR from *T. pardinum* and *P. involutus* increased the curiosity of mice more than the other extracts (Table 2).

With *per os* administration only the extracts of *P. involutus* increased dynamic activity in the mice (Table 3).

Spasmolytic Activity

The results after intraperitoneal administration of mushroom extracts revealed papaverine-like spasmolytic effects in mice that was demonstrated by a decreasing intestinal passability and diarrhea (Table 4).

In particular MSR from *P. involutus,* compared to control data, was active. It decreased intestinal passability by 57.39% at doses of 350 mg/kg. Extracts from *F. velutipes* showed no significant spasmolytic activity. WSR at a dose of 1000 mg/kg was more active (57.46%) than MSR (42.68%) at dose of 1200 mg/kg.

TABLE 1
Statistical Data of Analgesic Activity for Mice After Intraperitoneal Administration of Mushroom Residues (WSR, MSR)

Control/specimen	5–10 min	10–15 min	15–20 min	Total
C/I	$t = 0.21$	$t = 0.30$	$t = 0.72$	$t = 0.57$
C/II	$t = 1.77$	$t = 0.50$	$t = 1.78$	$t = 2.74$ $p < 0.05$
C/III	$t = 1.67$	$t = 0.38$	$t = 0.58$	$t = 0.72$
C/IV	$t = 1.19$	$t = 0.45$	$t = 0.20$	$t = 0.79$
C/V	$t = 2.06$	$t = 0.67$	$t = 0.48$	$t = 2.32$ $p < 0.05$
C/VI	$t = 1.80$	$t = 0.34$	$t = 1.23$	$t = 1.32$

C, control; t, Student's *t*-test; WSR: I, *F. velutipes;* II, *P. involutus;* IV, *T. pardinum;* MSR: III, *F. velutipes;* V, *P. involutus,* VI, *T. pardinum.*

TABLE 2
Statistical Data Concerning Central Nervous System Effects in Mice (Buasie's Test, Curiosity) After *Per Os* Administration of Mushroom Residues (WSR, MSR)

Control/Specimen	1 min	2 min	3 min	4 min	5 min
C/I	$t = 0.51$	$t = 0.41$	$t = 1.12$	$t = 0.82$	$t = 0.39$
C/II	$t = 3.14$; $p < 0.05$	$t = 2.22$	$t = 2.27$	$t = 2.53$; $p < 0.05$	$t = 1.04$
C/III	$t = 1.60$	$t = 2.16$	$t = 2.35$ $p < 0.05$	$t = 2.71$; $p < 0.05$	$t = 2.01$
C/IV	$t = 1.25$	$t = 1.47$	$t = 2.40$ $p < 0.05$	$t = 3.24$; $p < 0.05$	$t = 1.96$
C/V	$t = 1.61$	$t = 0.72$	$t = 1.21$	$t = 1.75$	$t = 0.84$
C/VI	$t = 1.65$	$t = 1.52$	$t = 2.48$	$t = 2.68$; $p < 0.05$	$t = 1.61$

C, control; t, Student's *t*-test; WSR: I, *F. velutipes;* II, *P. involutus;* IV, *T. pardinum;* MSR: III, *F. velutipes;* V, *P. involutus;* VI, *T. pardinum.*

TABLE 3
Statistical Data Concerning Central Nervous System Effects in Mice (Buasie's Test, Dynamic Activity) After *Per Os* Administration of Mushroom Residues (WSR, MSR)

Control/specimen	1 min	2 min	3 min	4 min	5 min
C/I	$t = 0.46$	$t = 1.04$	$t = 0.68$	$t = 0.54$	$t = 1.57$
C/II	$t = 0.36$	$t = 2.02$	$t = 1.88$	$t = 1.99$	$t = 3.53$; $p < 0.05$
C/IV	$t = 1.53$	$t = 0.16$	$t = 0.71$	$t = 1.31$	$t = 0.08$
C/V	$t = 0.62$	$t = 2.17$	$t = 1.28$	$t = 1.16$	$t = 2.57$; $p < 0.05$
C/III	$t = 0.65$	$t = 1.71$	$t = 1.10$	$t = 0.75$	$t = 1.33$
C/VI	$t = 0.95$	$t = 2.16$	$t = 0.07$	$t = 1.30$	$t = 0.08$

C, control; t, Student's *t*-test; WSR: I, *F. velutipes;* II, *P. involutus;* IV, *T. pardinum;* MSR: III, *F. velutipes;* V, *P. involutus;* VI, *T. pardinum.*

TABLE 4
Decreasing Intestinal Passability (in %) in Mice After Intraperitoneal Administration of Mushroom Residues

Species	Doses	*a*	*b*	*c*	*c(k) – c*	*D*
F. velutipes (MSF)	1200	16.9	53.2	32.0	23.8	–42.5
F. velutipes (WSF)	1000	10.6	45.1	23.8	32.1	–57.5
P. involutus (MSF)	350	12.2	51.1	23.8	32.1	–57.4
Control data	Carbon suspension	27	48.3	55.9	—	—

a, length of intestinal passability (in mm); *b,* total length of intestine (in mm); *c, a/b* × 100 (in %); *c(k),* passability within control mice; *c(k) – c,* diminution of passability (in %); *D,* diminution of passability (in %).

CONCLUSION

For the first time, extracts from fruiting bodies of three mushroom species—*F. velutipes, P. involutus,* and *T. pardinum*—were investigated for their pharmacological effects in mice, particularly analgesic, algogen, and spasmolytic activities, using various tests. Effects of mushroom extracts on the central nervous system, with increase of dynamic activity and curiosity of mice, were also observed. All tested doses of WSR and MSR from mushroom extracts provoked moderate changes in pharmacological tests, especially platform, traction, dynamic activity, catalepsy, piloerection, as well as Haffner's tests. WSR from fruiting body extracts, particularly *P. involutus,* showed a psychoanaleptic effect on the central nervous system, which was observed as augmentation of curiosity and dynamic

activity of animals. In the present experiments the extracts did not show stable analgesic activity. On the other hand, algogen activity of the mushroom extracts, particularly *P. involutus,* was observed. Significant papaverine-like spasmolytic activity with decreasing intestinal passability and diarrhea was found only for *P. involutus.* This was not described in the other tested mushroom, *F. velutipes.*

It will be therapeutically useful to check the revealed papaverine-like spasmolytic effect of mushroom extracts on pulmonary tissue to determine their potential antibronchospasmal or antiasthmal activity and their cardiovascular effect. A psychostimulating activity of the tested fungal extracts can be deepened by investigating the nature of neuromediators, noradrenergics, or serotoninergics.

Further mycopharmacological investigations of mushroom metabolites with medical importance is worth continuing to establish dose–effect relationships as well as the mechanism of action of more purified active compounds.

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