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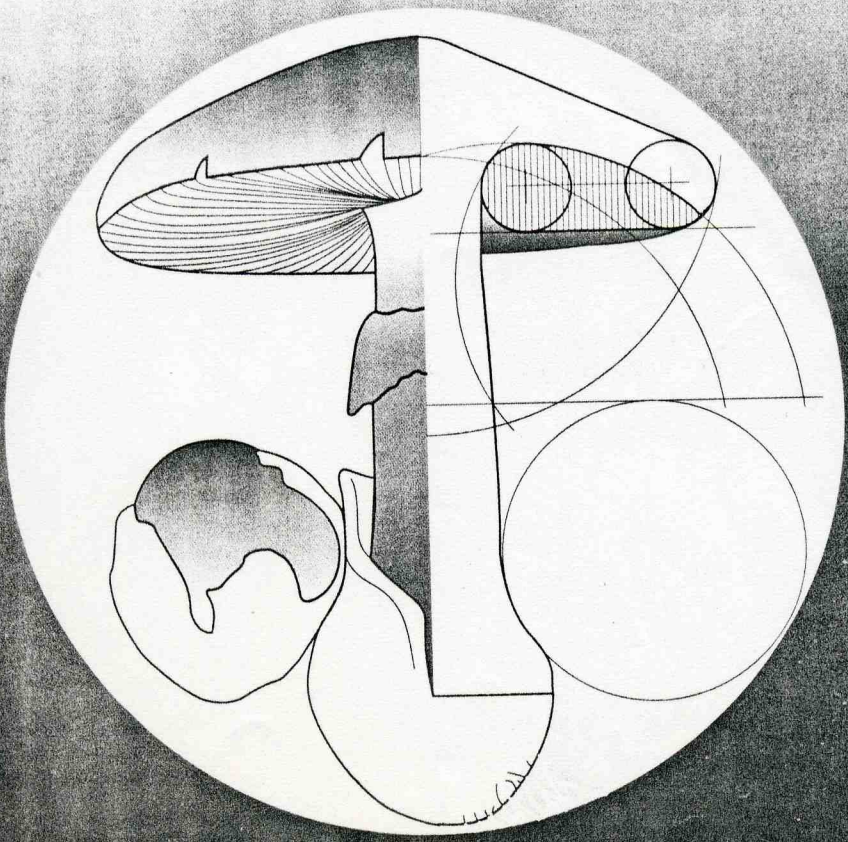

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Pharmacological activity of macroscopic fungi
Flammulina velutipes (Curt : Fr.) Sing., *Paxillus involutus* (Batsch : Fr.) Fr. and *Tricholoma tigrinum* Schaeff. *(Basidiomycotina)*

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ABSTRACT

Three species *Flammulina velutipes, Paxillus involutus* and *Tricholoma tigrinum (Basidiomycotina)* were investigated for pharmacological activities on mice. Especially, analgesic and spasmolytic activities of fungal extracts and their effect on central nervous system were reported. Both methanol-soluble and water-soluble residues separated from methanol extracts of fruit bodies of mushrooms were used. The fungal extracts didn't show particular analgesic effect. On the contrary, they demonstrated algogen activity, especially the *P. involutus*. Significant spasmolytic papaverine-like activity was also observed for *P. involutus*. This activity was revealed by decreasing intestinal passability and diarrhea. Particularly within *P. involutus* and *T. tigrinum* effect on central nervous system, with increase of dynamic activity and curiosity of mice, was observed.

INTRODUCTION

Pharmacological activity of macroscopic fungi *(Basidiomycotina, Ascomycotina)* is not sufficiently investigated. In view of recent advancements in biotechnology as well as myco-pharmacological studies, interest to fungal organisms as a natural source of valuable biological active metabolites with large spectrum of pharmacological actions (antibacterial, antifungal, antitumoral, hypoglycemic, fibrinolytic, neurotoxic, immunomodulating and others activities) has significantly increased (FUJIMOTO ET AL., 1986; 1991; 1992; OBUCHI ET AL., 1990; BECKER ET AL., 1994). The fungal polysaccharides [(1→3), (1→6)-a-D- or b-D-glucans] via the mechanism of cell recognition and activation of macrophages, are able to reinforce the immune response of organism against the antigens with a cytotoxic action (DE BERNARDI ET AL., 1983; KIHO ET AL.,1992; 1994; ZHANG ET AL., 1994; ZHUANG ET AL., 1994; 1996; KOBAYASHI ET AL., 1994; WANG ET AL., 1996; LIU ET AL., 1996). Immunomodulating and antiviral activities of lentinan (glucan from *Lentinula edodes*) against virus HIV were recently reported (SUZUKI ET AL., 1989). Fungal glycosides and terpenoides possess anti-inflammatory and vasodilatory effects (TAKAZAWA AND KASHINO, 1991; TAKAHASHI ET AL., 1992). Some fungal triterpenes and sesquiterpenes (neurotoxins) also provoke the paralyses of legs and nervous system and sometimes become lethal (FUJIMOTO ET AL., 1986; 1991; 1992; BADALYAN ET AL., 1995).

Thus, macroscopic fungi are perspective objects for biotechnological and pharmacological research. Many of them can be used for treatment of diseases, such as diabetes, cancer, as well as bacterial and fungal infections.

This paper aims at presenting the pharmacological activities of *F. velutipes, P. involutus* and *T. tigrinum*. It is well known, that the poisonous mushroom *P. involutus* provokes gastro-intestinal and cardio-vascular troubles, often with hemolytic effect (Paxillus Syndrome). *T. tigrinum* can cause disturbance of nervous system and gastro-intestinal colic (BRESINSKY AND BEST, 1990). Data concerning pharmacological activities, such as antitumor and fibrinolytic activities of edible mushroom *F. velutipes* can also be detected (WATANABE ET AL., 1964; HIRANO ET AL., 1987).

MATERIAL AND METHODS

Fungal material. The fruit bodies of *F. velutipes, P. involutus* and *T. tigrinum* were collected in Armenia, Germany and France, respectively. Freshly gathered mushrooms were identified by the authors.

Preparation of extracts and their fractionation. Finally, powdered fruit bodies were extracted by methanol (×5, 100ml) with magnetic agitation at room temperature as follows (Fig. 1).

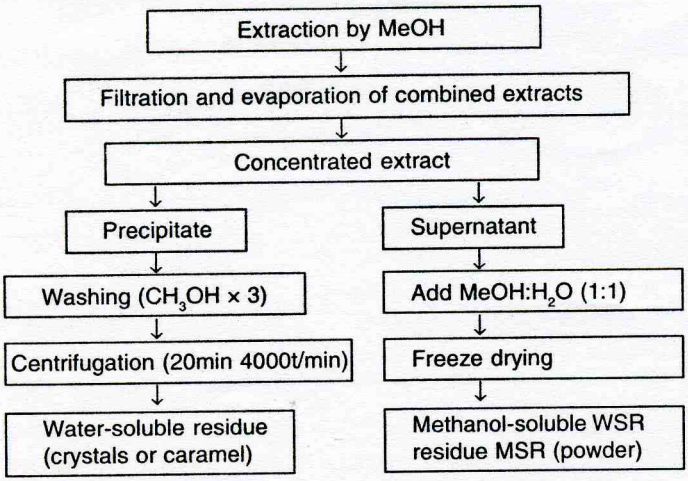

Fig. 1. Extraction and purification of fungal extracts.

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

Experimental conditions. Adult EOPS Swiss mice, from 5 to 6 weeks old, weighing 20-26g were used. The animals were housed 3 or 5 per cage (25×45×15cm). The adaptation of the animals has taken place at room temperature (22°C) and relative humidity 60±10%. The mice were set on a 12 hour light/dark cycle. They had free access to commercial pelleted diet (UAR AO4) and tap water *ad libidum.*

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

Pharmacological tests and doses. To estimate pharmacological activity and toxicological evaluations of fungal extracts various pharmacological tests, such as the platform, traction, dynamic activity, catalepsy tests, as well as flexion, posture, pineal, corneal and Haffner' reflexes were used. Tests were recorded twice in the first hour and every hour during the following 6 hours, on day 5, 10 and 14. The animals were weighed at day 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. tigrinum).* 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. tigrinum)*. Each dose was tested on 3 mice. Calculations of the pharmacological results were done by 4 possible ways: 0/=0%; 1/3=33%; 2/3=66% and 3/3=100%.

Analgesic activity of fungal extracts *in vivo* was detected on mice in the same experimental condition using Coster's method. According to this method, 1% acetic acid (0.2 ml/20g) was injected i. p. as an algogen factor provoking pain and contractions of dorso-abdominal muscles. Primarily, injected (i. p.) fungal 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. tigrinum* - 800 mg/kg each. Analgesic or algogen activity was calculated by numbers of colic using following formula: $P\%=100\times(K-C)/K$, where *K*- number of cramps within control animals; *C* - number of cramps within animals after administration of fungal extracts.

Effect on central nervous system. The extracts from three species was investigated in the same experimental condition using *Buasie*'s test. It permits to study dynamic activity and curiosity of the animals during the 5 min following 45 min of *per os* administration of fungal 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. tigrinum* - 1200 mg/kg; MSR from *F. velutipes* 900 mg/kg, *P. involutus* - 1200 mg/kg and *T. tigrinum* - 350 mg/kg. Each dose was tested on 5 mice.

Spasmolytic activity. Fungal extracts was detected for spasmolytic activity after *per os* administration of carbon suspension (10 ml/mg), that allows us to observe the movement of intestinal content. 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 doses 3 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* - length of intestinal passability; *b* - total length of intestine; *c(k)* - passability within control mice; *c(k)-c* - diminution of passability; *D* - passability in per cent.

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

RESULTS AND DISCUSSION

Estimation of pharmacological activity. No death or significant pathology was seen in the animals after single i. p. or *per os* administration of fungal residues at doses ranging from 350-2000 mg/kg. These results were confirmed by the animal weight at different doses for the same period of time (unpublished data). Autopsy of animals sacrificed on day 14, doesn't reveal digestive, pulmonary or others significant changes. Also, no significant changes of Hafner's reflex were observed. However, extracts from *P. involutus* and *T. tigrinum* defined piloerection, increasing or decreasing dynamic activity within 66% of mice, as well as diarrhea. These data were confirmed by complementary pharmacological tests, such as curiosity and spasmolytic effect of tested residue. It was reported, that pharmacological troubles provoked by *per os* administration of mushroom's extracts were more significant than troubles due to i. p. administration. At the same time, it was mentioned that the WSR from fungal extracts of the tested mushrooms was more active pharmacologically than MSR. Previous chemical analyses of these fungal extracts have shown that the WSR contain more polysaccharides, sugars, polyols, terpenoides, while the MSR contain alkaloids, amino acids, nynhidrine-positive, indolic compounds, as well as phenolic acids (Badalyan, 1998).

Analgesic activity. Tested extracts from fruit bodies of *F. velutipes, P. involutus* and *T. tigrinum*

did not show stable analgesic effect. However, during the first 5 minutes of the experiment, reinforcement and augmentation in the number of cramps were observed, particularly after using the residues (WSR, MSR) from *P. involutus*. The statistic data ($p<0.05$) show an average number of colic during the 20 minutes of experiment compared to control data grown-up. After a few minutes the situation was stabilized (table 1).

Table. 1. Statistic data of analgesic activity for mice after i. p. administration of fungal extracts

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; WSR: I - *F. velutipes*; II - *P. involutus*; IV - *T. tigrinum*;
MSR: III - *F. velutipes*; V - *P. involutus*; VI - *T. tigrinum*.

The data of total number of colic calculated by above mentioned formula in comparison with control data 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. tigrinum*), respectively. The results sown, that i. p. administration of MSR provoked more augmentation of number of colic within animals by comparison with control data, than i. p. administration of WSR.

Effect on central nervous system. Per os administration of all tested fungal specimen during 5 min of experiment increases dynamic activity and curiosity of animals. The WSR from *T. tigrinum* and *P. involutus* increase the curiosity of mice more than other specimen (table 2).

Table 2. Statistic data concerning central nervous system (curiosity) effect for mice after *per os* administration of fungal extracts

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; WSR: I - *F. velutipes*; II - *P. involutus*; IV - *T. tigrinum*;
MSR: III - *F. velutipes*; V - *P. involutus*; VI - *T. tigrinum*.

After *per os* administration only both MSR and WSR from methanol extract of fruit bodies of *P. involutus* increase dynamic activity in the mice (table 3).

Table 3. Statistic data concerning central nervous system (dynamic activity) effect for mice after *per os* administration of fungal extracts

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; WSR: I - *F. velutipes*; II - *P. involutus*; IV - *T. tigrinum*;
MSR: III - *F. velutipes*; V - *P. involutus*; VI - *T. tigrinum*.

Spasmolytic activity. The results after i. p. administration of fungal extracts revealed papaverine-like spasmolytic effect for mice that is demonstrated by a decreasing intestinal passability and diarrhea (table 4).

Table 4. Decreasing of intestinal passability (in %) for mice after i. p. administration of fungal extracts

Species	Doses	*à*	*b*	*c*	*ñ(k)-ñ*	*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); $c = a/b \times 100$ (in %); ***c(k)*** - passability within control mice; ***c(k)-c*** - diminution of passability (in %); ***D*** - diminution of passability (in %%)

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

CONCLUSION

For the first time, extracts from fruit bodies of three species *F. velutipes, P. involutus* and *T. tigrinum* were investigated for pharmacological, particularly analgesic, algogen and spasmolytic activities using different tests. Effect of fungal extracts on central nervous system was observed as well. All tested doses of water-soluble and methanol-soluble residues from fungal extracts provoked moderate changes in pharmacological tests, especially platform, traction, dynamic activity, catalepsy, piloerection, as well as Haffner's tests. In our experiments the extracts didn't show stable analgesic activity. On the other hand, algogen activity of mushroom's extracts,

particularly *P. involutus* was reported. WSR from fruit bodies' extracts, particularly *P. involutus* showed an effect on central nervous system, which was observed by augmentation of curiosity and dynamic activity of animals. Significant papaverine-like spasmolytic activity with decreasing intestinal passability and diarrhea was only found for *P. involutus*. This was not described in other tested mushroom *F. velutipes*.

Further pharmacological studies of extracts from fruit bodies of *F. velutipes, P. involutus* and *T. tigrinum* should be carried to check other characteristics of the above discussed activities by using more purified substances.

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