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Toxicological screening of seven Nigerian mushrooms

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Toxicological studies were carried out on seven Nigerian mushrooms, namely, *Chlorophyllum molybditis* (Mayer ex. fr.) Masse, *Cortinarius melliolens* Fries, *Lentinus subnudus* Berk, *Pleurotus tuber-regium* (Fries) Singer, *Termitomyces robustus* (Beeli) Heim, *Tricholoma lobayensis* Heim and *Volvariella esculenta* (Mass) Singer. Amatoxin spot test and chromatographic screening of the mushrooms revealed the absence of amatoxins and phallotoxins. None of the mushroom extracts tested killed the experimental rats. In fact, some of the rats which fed on or were injected intraperitoneally which mushroom extracts showed some significant gain in dry weight. The significance of these findings is discussed in relation to consumption of these mushrooms.

INTRODUCTION

The consumption of poisonous mushrooms, also called toadstools, causes discomfort such as stomach upset, diarrhoea, emesis, intoxication, dizziness, convulsion and, in the case of highly toxic ones, death within 2 to 5 days after mushroom ingestion (Wieland, 1968; Gray, 1973). Species of *Amanita, Galerina* and *Phallus* genera constitute the vast majority of poisonous mushrooms and examples are *A. phalloides. A. verna, G. maginata, G. venerata* and *P. aurantiacus* (Gray, 1973; Oso, 1976). *Coprinus* species are usually avoided because of their toxicity especially when taken with alcohol (Walton, 1964; Gray, 1973; Oso, 1975).

The toxic components of poisonous mushrooms have been identified as amatoxins, phallotoxins, gyromitrin, orellanine, muscarine, tricholomic acid, ibotenic acid, muscinol, psilocybin, psilocin and tetraethyl thiuram disulphite (Wieland, 1968; Benedict, 1972; Wieland & Wieland, 1972; Baku *et al.*, 1980; Saupe, 1981). When mushrooms containing amatoxins, phallotoxins, gyromitrin, orellanine, muscarine and tricholomic acid are eaten, death usually results, whereas eating mushrooms that contain ibotenic acid, muscinol, psilocybin, psilocin and tetramethyl thiuram disulphite causes some of the symptoms listed above, but not death (Benedict, 1972; Wieland & Wieland 1972).

In toxicological screening of mushrooms, a lot of emphasis is placed on the presence of amatoxins and phallotoxins because *Amanita* species that contain these toxins accounted for 90% of the deaths that resulted from mushroom poisoning between 1924 and 1961 (Buck, 1961). In this study, seven Nigerian mushrooms were screened for toxins because these mushrooms are very common in Nigerian vegetation and are sold in local markets. To our knowledge only *T. robustus* and *C. molybditis* have so far been examined for amatoxins and phallotoxins (Alofe, 1985).

MATERIALS AND METHODS

Collection of mushrooms

Fruit bodies of *Chlorophyllum molybditis* (Mayer ex. Fr.) Masse, *Cortinarius melliolens* Fries, *Tricholoma lobayensis* Heim, *Volvariella esculenta* (Mass.) Singer and *Termitomyces robustus* (Beeli) Heim were collected from the field, while those of *Pleurotus tuber-regium* (Fries) Singer and *Lentinus subnudus* Berk were harvested from daily watered, soil-planted sclerotium and logs of *Spondias mombin* Linn, respectively, in the Departmental garden. The harvested fruit bodies were dried at 80°C for 2 days and powdered in a Moulinex blender.

Toxin extraction

Powdered samples (500 mg) were defatted with 30 ml of light petroleum for 3 h in a Soxhlet extractor. The insoluble residue was dried in the oven and extracted with 30 ml of boiling methanol for 3 h in a Soxhlet extractor (Horgen *et al.*, 1976; Yocum & Simons, 1977). The resulting extract was evaporated to dryness using a rotary evaporator, redissolved in 2 ml of methanol and

centrifuged for 30 min using a bench centrifuge. The supernatant was used as a methanolic extract.

Amatoxin spot test

This was done according to the method of Wieland & Faulstich (1978). A 30 μl portion of the methanolic extract was spotted on newsprint, and the newsprint was exposed to the fumes of concentrated hydrochloric acid. The development of a blue colour within 20 min confirmed presence of amatoxin.

Qualitative test for amatoxins and phallotoxins

This was done according to the method of Block *et al.*, (1955*a*), Wieland (1968) and Yocum & Simons (1977). Amatoxin and phallotoxin solutions were prepared by dissolving 1 mg of α-amanitin and 4 mg of phalloidin in 2 ml of sterile distilled water. Portions (30 μl) of the methanolic extracts from the seven mushrooms, amatoxin and phallotoxin solutions were loaded on to a Whatman No. 1 chromatographyic paper, which was developed descendingly for 12 h, using butanone–acetone–water (30:3:5, v/v) as running solvent. The dried chromatogram was dipped in a 1% solution of cinnamaldehyde in methanol and immediately exposed to the vapour of fuming concentrated hydrochloric acid (blue or purple spots indicate phallotoxin; violet spots indicate amatoxin; while orange, yellow, brown and pink spots are of no significance).

Oral toxicity test of mushroom water extracts

The powdered mushroom sample (5 g) was boiled in 250 ml of distilled water for 30 min and filtered (Block *et al.*, 1955*b*). A 25 ml aliquot of the aqueous mushroom extract and 12 g of rabbit pellets were fed to a weighed 6-week-old rat on a daily basis for 10 days. Similarly, a weighed 6-week-old rat given 25 ml of distilled water and 12 g of rabbit pellets on a daily basis for 10 days was used as the control. At the end of 10 days both the experimental and control rats were weighed. The experiment was replicated four times. During the 10 day experimental period, the rats were observed for clinical symptoms and death. Moreover, after the 10 day study period, the urine of all the rats was tested for protein, using the biuret test. This is because amatoxin-containing mushrooms usually damage the kidney and cause protein to appear in the urine (Wieland, 1968). The rats were then killed by exposure to chloroform fumes, weighed and dried in the oven at 80°C for 4 days for dry weight determination.

Intraperitoneal toxicity test of mushroom methanolic extracts and commercial mushroom toxins

This was performed according to the method of Block *et al.*. (1955*a,b*). Methanolic extracts of powdered samples of the seven mushrooms species were prepared as described earlier. The methanolic extract was evaporated to dryness with a rotary evaporator and redissolved in 1 ml of sterile distilled water. One milligram of α-amanitin and 4 mg of phalloidin were each dissolved in 1 ml of sterile distilled water, respectively, to serve as controls. Weighed 6-week-old rats were each injected intraperitoneally with 0·5 ml of the mushroom extract. Three sets of controls were employed using 6-week-old rats. The first control was injected intraperitoneally with 0·5 ml of α-amanitin solution, the second with 0·5 ml of phalloidin and the third with 0·5 ml of sterile distilled water. During the 35 days of experimental study, the rats were carefully observed for clinical symptoms or death and the weights of rats that died during the study were recorded. The surviving rats were weighed at the end of the 35 days study period.

Statistical analysis

The data obtained were analysed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

RESULTS

The amatoxin spot test of the mushroom extracts revealed the absence of amatoxin because no blue spot was observed. Chromatographic tests of the mushrooms for amatoxins and phallotoxins were also negative because yellow or orange spots were seen. The phalloidin solution used as the standard gave a purple colour, while the α-amanitin solution showed a violet colour. From these tests, it is clear that all the mushrooms tested were free from amatoxins and phallotoxins.

Rats that were orally given aqueous extracts of the various mushrooms for 10 days did not die and the biuret test of their urine for protein was negative. No significant clinical symptoms were observed. However, significant increases in body weights ($P = 0{\cdot}01$) were noticed in rats given aqueous extracts of *C. melliolens, P. tuber-regium, T. robustus. T. lobayensis* and *V. esculenta* (Table 1). Similarly, significant dry weight increases ($P = 0{\cdot}01$) were noticed in rats treated with *C. melliolens, T. robustus, T. lobayensis* and *V. esculenta* (Table 1).

Rats injected with phalloidin and α-amanitin died on the first and second day, respectively, whereas rats injected with distilled water and mushroom extracts did not die during the 35 day study period (Table 2). Phalloidin and α-amanitin injected rats showed loss in body weight, while rats injected with extracts of *C. melliolens, L. subnudus. P. tuber-regium. T. robustus, T. lobayensis* and *V. esculenta* showed a gain in body weight.

DISCUSSION

The qualitative test performed showed that none of the seven Nigerian mushrooms contained α-amanitin or

Table 1. Weights of rats fed with mushroom aqueous extracts

Mushroom	Average increase in body wt. at the end of study period(g)	Average dry wt. of rats at the end of study period(g)
C. molybditis	17·8d	22·4c
C. melliolens	36·7a	26·2b
L. subnudus	21·3cd	21·3c
P. tuber-regium	24·4c	22·3c
T. robustus	37·5a	26·7b
T. lobayensis	34·3ab	30·6a
V. esculenta	31·0b	27·1b
Control	17·5d	20·8c

Means followed by the same letter(s) within any column are not significantly different at $P = 0{\cdot}01$ by Duncan's multiple range test.

Table 2. Clinical observations of rats given interperitoneal injection of mushroom extracts and pure mushroom toxins

Mushroom species/ toxin type	Average increase/ decrease in body wt. at the end of study period or at the time of death(g)	Time of death
C. molybditis	23·5c	No death
C. melliolens	40·0a	No death
L. subnudus	32·5b	No death
P. tuber-regium	36·5a	No death
T. robustus	30·0b	No death
T. lobayensis	37·5a	No death
V. esculenta	29·0b	No death
α-Amanitin (control)	–3·5d[a]	Died on the 2nd day
Phalloidin (control)	–2·0d	Died on the 1st day
Distilled water (control)	22.5c	No death

[a]Indicates loss in weight
Means followed by the same letter(s) are not significantly different at $P = 0{\cdot}01$ by Duncan's multiple range test.

phalloidin. Similarly, none of the mushroom extracts given to the rats orally and intraperitoneally caused death or elicited any adverse clinical symptoms during the 35 day experimental period. The rats were healthy and had significant gains in body weight except rats given *C. molybditis, P. tuber-regium* and *L. subnudus* extracts (Tables 1 and 2). In contrast, rats injected with α-amanitin and phalloidin died within 2 days (Table 2). In the toxicological screening of mushrooms, a lot of emphasis is placed on the presence of amatoxins and phallotoxins because these are known to have caused 90% of the reported deaths (1924–1961) from poisoning by *Amanita* species (Buck, 1961). From these findings, it appears that the Nigerian mushrooms tested in the present study are nontoxic. This result is consistent with that obtained by Alofe (1985) for *C. molybditis.* Block *et al.* (1955*b*), likewise screened 46 mushroom species, including 13 amanitas, for amatoxins and phallotoxins and detected toxins in only *Amanita verna and A. tennuifolia.* Yocum & Simons (1977) investigated *A. phalloides, A. bisporegera, A. verna, A. virosa* and *A. rubescens* and detected toxins in all the amanitas except *A. rubescens.*

In the present study, none of the rats given mushroom extract excreted protein in their urine. This means that their kidneys were not affected by the mushroom extracts; this suggests the absence of amatoxins in the mushroom tested. Amatoxins are known to damage convoluted tubules and thereby prevent ultrafiltration (Wieland, 1968). From our present study, amatoxins and phallotoxins were absent in the seven Nigerian mushrooms investigated. This implies that the seven mushrooms screened are edible. Hence *L. subnudus, P. tuber-regium, T. robustus, T. lobayensis* and *V. esculenta,* which are eaten commonly and also used for ethnomedicinal purposes (Oso, 1975, 1977) are considered edible because they are safe. Moreover, there has not been any reported case of toxicity arising from the comsumption of these mushrooms. As for *C. melliolens* and *C. molybditis,* caution should be exercised in eating them until further proofs of their edibility are established. According to Zoberi (1973), some forms of *C. molybditis* are edible, while others are regarded as poisonous. It has been suggested that its toxicity depends on climatic and habitat factors. In Nigeria, both *C. melliolens* and *C. molybditis* are avoided by mushroom collectors and consumers.

In conclusion, *L. subnudus, P. tuber-regium, T. robustus, T. lobayensis* and *V. esculenta,* which historically have been known to be edible and in the present study are confirmed to be nontoxic, are, therefore, recommended as edible mushrooms.

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