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Potentiation of ethanol by *Coprinus atramentarius* in mice

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Assessment of toxic manifestations and sleeping times in mice shows that the mushroom *Coprinus atramentarius* potentiates the action of ethanol when administered orally from 3 to 16 hr before a sub-lethal dose of ethanol. Administration of this mushroom 24 hr before, immediately after or 3 hr after the ethanol causes no potentiation. No similar effect was observed with *Coprinus comatus*.

THERE are about 75 North American species of *Coprinus*. One of the best known is *Coprinus atramentarius* (inky cap), a black-spored mushroom which is generally considered edible. Reports in the literature indicate a toxic reaction may result when this species is consumed with ethanol, and warn that ethanolic beverages should not be taken shortly before, during or after ingestion of the mushroom (Buck, 1961; Groves, 1962; Tyler, 1963). The related species *C. comatus* (shaggy mane) has also been incriminated (Zeitlmayr, 1955). On the other hand, Krieger (1911) and Child (1952) agree that *C. atramentarius* does not sensitize man to ethanol. Human idiosyncrasies, mode of preparation, and timing of consumption in relation to ethanol ingestion appear to influence the toxicity (Tyler, 1963). Attempts have been made to identify the toxic principle of *C. atramentarius*, and Simandl & Franc (1956) claim the isolation of disulfiram (Antabuse) which inhibits the *in vivo* oxidation of acetaldehyde. These findings have not been confirmed by others (List & Reith, 1960; Wier & Tyler, 1960). Four cases of poisoning attributed to inky caps and ethanol were recently reported by Reynolds & Lowe (1965).

We have investigated whether potentiation of ethanol by two *Coprinus spp.* could be demonstrated in mice.

Experimental

MUSHROOMS

Coprinus atramentarius (Bull. ex Fr.) Fr. (4·3 kg) and *C. comatus* (Mull. ex Fr.) S. F. Gray (0·9 kg) were collected on Ottawa lawns between August and October 1966. The samples were cleaned and frozen (−25°) within ½ hr of collection, freeze-dried within 5 days and powdered in a Wiley mill to pass a 100 mesh sieve. The dehydrated powder was stored at −25°. The average water content of a fresh sample of either species was 92%.

Sample preparation. Raw mushroom powder suspensions in a 0·25% aqueous tragacanth solution were freshly prepared. Cooked mushroom samples were obtained by reconstituting the mushroom powder with an appropriate amount of water and then boiling the mixture for ½ hr with stirring. After cooling, tragacanth was added. Animals were fed by stomach tube (2·4 or 2·5 ml suspension per 30 g mouse).

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ETHANOL

Solutions of ethanol 30% w/v in water were administered by stomach tube. The doses selected were 5 or 6 g/kg. Lower doses did not affect the righting reflex and appreciably higher doses resulted in death.

ANIMALS

Non-fasted male albino mice (Quebec Farm) approximately 30 g were used. Their diet consisted of Master Fox Chow and water *ad lib.* All animals were randomly assigned to the experimental groups. They were observed for physiological symptoms in cages containing groups of 10. In the sleeping time experiment they were placed on tables with marked squares as soon as they fell asleep.

DEFINITIONS

Scores. The observed signs of ethanol intoxication were assigned scores, as follows: 4 death; 2 loss of righting reflex; 1 ataxia; ½ slight ataxia, other impairment (e.g., eyes closed, sedated); 0 normal. The total score in each experimental group was obtained by addition of the individual scores.

Induction time is the time interval between administration of ethanol and loss of righting reflex.

Sleeping time is the time interval between loss and return of righting reflex.

Results

The toxicity of raw mushroom suspensions was explored in preliminary experiments. Ingestion of very thick suspensions of *C. atramentarius* and *C. comatus*, up to 10·1 g/kg, had no visible effect other than sedation.

EXPERIMENT I

This was a preliminary investigation of the influence of the time between feeding mushroom suspensions and a sublethal dose of ethanol on toxic effects. Raw *C. atramentarius* and *C. comatus* (4·5 g/kg) were fed to groups of 10 mice. Ethanol (5 g/kg) was administered 3 hr before, immediately after, and 3, 6, 16 and 24 hr after mushroom feeding. Control groups of 10 animals were fed only ethanol, *C. atramentarius* or *C. comatus*. The animals were observed for 40 hr and their scores recorded. In the mushroom control groups the score was zero for the entire period. Scores of the ethanol control group were 11 (1 hr), 3·5 (3 hr) and zero after 4 hr. Ethanol + *C. atramentarius* groups, given the ethanol 3 or 6 hr after the mushroom, scored higher than the ethanol control group. Groups given the ethanol immediately or 16 hr after the mushroom had higher scores than the ethanol control group 4 hr after ethanol administration. Groups administered ethanol 3 hr before, or 24 hr after the mushroom meal had scores similar to the ethanol control group. Animals given *C. comatus* and ethanol showed only minor score differences from

the ethanol controls so further experimentation with this species was discontinued. No deaths occurred in this experiment; the main symptoms noted were ataxia, loss of righting reflex and sedation.

EXPERIMENT II

This was to assess that time interval between feeding of *C. atramentarius* and ethanol leading to the highest score. Also the action of cooked and raw mushroom was compared.

Raw and cooked *C. atramentarius* (8 g/kg), were administered alternately to groups of 10 mice, six groups receiving each preparation. Two groups (one fed raw and the other cooked mushroom) were used as controls and ethanol (5 g/kg) was administered to each of the other groups

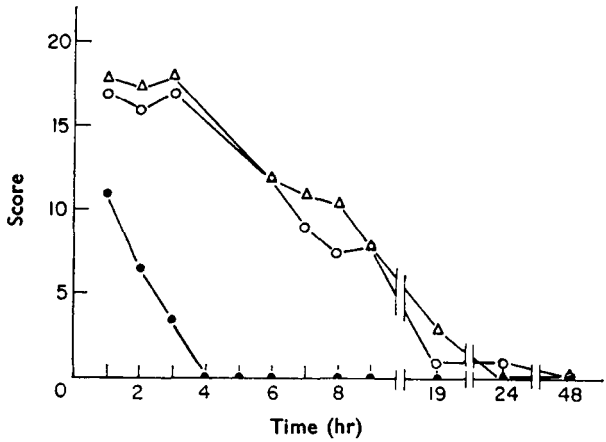

FIG. 1. Observed score vs. time (hr) after ethanol administration in mice given (●) ethanol only, (○) raw mushroom + ethanol (after 4 hr), and (△) cooked mushroom + ethanol (after 4 hr).

after 4, 6, 8, 10 and 12 hr, respectively. Another control group of 10 mice received ethanol only. The animals were observed for 48 hr and their scores recorded. The group dosed with ethanol 4 hr after the mushroom preparation showed the highest score. The scores of groups administered cooked and raw mushrooms were nearly identical (Fig. 1). Raw mushrooms and the 4 hr interval between mushroom and ethanol administration were therefore used in further work.

EXPERIMENT III

Sleeping times were measured with 120 animals in groups of 10. Six groups were given 6 g/kg of ethanol only. The other six groups were administered 8 g/kg of raw *C. atramentarius* preparation and, after 4 hr, 6 g/kg ethanol. Ethanol groups and the mushroom + ethanol groups were fed alternately. The elapsed time for feeding was 6 min per group. Results showing the sleeping time and the incidence of sleeping in both experimental groups are given in Table 1. The incidence of sleeping in

TABLE 1. SLEEPING TIME AND INCIDENCE OF SLEEPING AFTER ADMINISTRATION OF ETHANOL AND *C. atramentarius*

Treatments	Number of animals sleeping	% of animals sleeping	Deaths	Average induction time (min)	Average sleeping time (min)
Ethanol	19/60	32	0	30 (6–66)*	203 (39–670)
Mushroom (raw) + ethanol (4 hr later)	54/60	90	2†	28 (5–52)	394‡ (104–775)

* Figures in parentheses are ranges.
† One death before and one after regain of righting reflex.
‡ Animal which died before regain of righting reflex not included.

the mushroom ethanol-treated mice increased markedly over those given ethanol alone (P of χ^2 <0·005). Although the averages of the induction times in the two groups were close, the average sleeping time in the mushroom + ethanol group was nearly double that of the control group. An analysis of variance for log induction time and log sleeping time is

TABLE 2. ANALYSIS OF VARIANCE OF SLEEPING TIME

Source	d.f.	Mean squares: Induction time	Mean squares: Sleeping time
Treatments ..	1	0·020	1·523*
Within ..	70	0·086	0·051

* P <0·01.

given in Table 2. It shows that the sleeping times are significantly different for the two treatments (P <0·01), but that induction time is not.

Discussion

The time factor of the *C. atramentarius*-ethanol syndrome in man is controversial. Buck (1961) states that nitritoid symptoms are caused by this mushroom if ethanolic beverages are drunk before, during or after its ingestion. List & Reith (1960) who also cited older European literature on poisoning cases with *C. atramentarius*, reported toxic effects in one person after a 250 g (about 20 g dry weight) meal of cooked mushrooms and consumption of ethanolic beverages (approximately 28 ml ethanol) immediately after the meal; a glass of beer after 16 hr; and a glass of wine after 24 hr. The toxic effect was observed one day after the mushroom meal. However, two persons who ingested the same amount of raw mushroom and ethanol had no adverse effects. Further poisoning cases have been cited by Wier & Tyler (1960).

We found that a toxic reaction in mice could be demonstrated best when ethanol was given 3 to 6 hr after mushroom feeding. It was less manifest with simultaneous administration or with a 16 hr feeding interval. After a 24 hr interval or with reversal of the feeding order the toxic effect as practically absent.

Sleeping times have been used extensively in studies of the combined effects of ethanol and drugs in animals (Aston & Cullumbine, 1959;

Svedin, 1966) but to our knowledge no comparable data on the *Coprinus*-ethanol potentiation in animals have been reported. This reaction does not appear to be unique for *C. atramentarius* since *Boletus luridus* (Zeitlmayr, 1955) and Morels (Groves, 1964) have also been reported to exert this effect.

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