J. Pharm. Pharmac., 1969, **21**, 176–179 *Received September* 16, 1968

Effect of *Coprinus atramentarius* on the metabolism of ethanol in mice

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The ethanol-soluble material extracted from *Coprinus atramentarius* was more toxic to mice, as measured by the LD50 and potentiation of the ethanol-induced sleeping time, than the whole mushroom or the residue remaining after ethanol extraction. Also, the ethanol-soluble fraction when fed to mice 4 h before administration of ethanol markedly increased the blood acetaldehyde and ethanol levels.

Previously Genest, Coldwell & Hughes (1968) reported that the fungus, *Coprinus atramentarius* (inky cap), potentiated the toxic effects of ethanol in mice but that *Coprinus comatus* (shaggy cap, lawyer's wig) did not. The toxic and ethanol-potentiating effects in mice of *Coprinus atramentarius* has now been investigated and also its ethanol-acetic acid soluble and ethanol insoluble fractions.

EXPERIMENTAL

Coprinus atramentarius (Bull. ex Fr.) Fr. (20 lb) was collected on Ottawa lawns during the autumn of 1967 and dried as described by Genest & others (1968). The dried powdered fungus (IC/A) was percolated in 200 g batches with 3 litres of 96% ethanol at a rate of 2–4 ml/min. After adding 0·72 ml of 50% sulphuric acid to ensure retention of volatile bases (List & Reith, 1960), the percolate was evaporated at 30° *in vacuo* in a rotary evaporator to 200–400 ml. The marc was treated twice with 200 ml each of 10% acetic acid on a water bath at 80°. After filtration, the marc was dried at 60° (IC/B). The ethanol and acetic acid extracts were combined, the ethanol completely expelled in a rotary evaporator and the remaining water removed by freeze-drying. The residue (IC/C) represented 43% of the whole mushroom. The pH of aqueous suspensions of samples of IC/A, IC/B and IC/C were 6·6, 4·7 and 4·8, respectively. Freshly prepared suspensions of either one of fractions IC/A, B or C (1 g/10 ml) in 0·25% aqueous gum tragacanth were fed to mice (2·5 ml/30 g body weight) by stomach tube.

Non-fasted male albino mice of Connaught strain weighing 27 ± 4 g were assigned randomly to the experimental groups. They were housed in all-metal cages, 10 or 12 per cage with free access to Master Fox Chow and water in a room at 72° ± 2° F. They were acclimatized to the environment of the animal quarters at least 10 days before beginning the experiments.

Solutions of 30% w/v ethanol in water were administered by stomach tube. A non-lethal dose of 6 g of ethanol in solution per kg was given; this was sufficient to cause loss of the righting reflex in about 20% of the animals.

RESULTS

Induction and sleeping times

These were measured as described by Genest & others (1968), in 270 mice in groups of 10, and treated as follows.

IC/A 8 g/kg, 4 h later water 0·5 ml/30 g (10 mice); IC/B 4·6 g/kg, water 0·5 ml/30 g (10 mice); IC/C 3·4 g/kg, water 0·5 ml/30 g (10 mice); 0·25% gum tragacanth, 2·5 ml/30 g, 4 h later ethanol 6 g/kg (60 mice); IC/A 8 g/kg, ethanol 6 g/kg (60 mice); IC/B 4·6 g/kg, ethanol 6 g/kg (60 mice); IC/C 3·4 g/kg, ethanol 6 g/kg (60 mice). Both IC/B and IC/C were given at doses equivalent to the amount of each present (% w/w) in IC/A (8 g/kg). The results are summarized in Table 1.

Table 1. *Induction time (I.T.), sleeping time (S.T.) and incidence of sleeping after administration of ethanol alone and with* C. atramentarius *and its ethanol-soluble and insoluble fractions to mice*

	Treatments			
Measurement	Ethanol (a)	IC/B + ethanol (b)	IC/A + ethanol (c)	IC/C + ethanol (d)
Number of mice sleeping ..	12/58	28/59	47/59	54/59
% of mice sleeping	21	47	80	92
Probability of χ^2_Y	<0·001 (a, b)	<0·001 (b, c)	<0·07 (c, d)	
I.T.—median and range (min) ..	40 (16–97)	35 (5–109)	33 (9–187)	30 (2–230)
S.T.—median and range (min) ..	163 (15–258)	236 (98–544)	438 (31–945)	639 (22–1092)
Probability† ..	<0·02 (a, b)	<0·05 (b, c)	<0·01 (c, d)	

† Calculated by the Wilcoxon rank sum test (Wilcoxon, 1945).

The incidence of sleeping among the mushroom + ethanol-treated mice was increased significantly over that observed in mice treated with ethanol alone confirming our earlier observation (Genest & others, 1968). Also, the incidence of sleeping was highest after treatment with IC/C + ethanol and higher in the IC/A + ethanol group than in the IC/B + ethanol treated animals. IC/A, B, or C administered without ethanol did not cause loss of the righting reflex. The induction times exhibited no significant differences between treatments. However, the sleeping times were increased significantly when IC/A, B or C were given 4 h before administration of the ethanol, the greatest effect being caused by IC/C, followed by IC/A and IC/B. The results on incidence of sleeping and on sleeping time strongly indicate that the toxic component(s) of *C. atramentarius* are at least partially extracted with ethanol-acetic acid.

LD50 of IC/C

The ethanol-soluble fraction of *C. atramentarius* (IC/C) was administered orally at 0, 6·5, 8·1, 10·2, 12·7 and 15·9 g/kg. The numbers of deaths in 24 h were respectively 0/10, 0/12, 2/12, 4/12, 9/12, 9/12. The LD50 with 95% confidence limits as estimated by the method of Litchfield & Wilcoxon (1949) was 11·2 (9·4–13·3) g/kg. The animals exhibited clonic convulsions immediately before death. The LD50 of IC/A and IC/B could not be estimated because the thickness of the suspensions prevented administration of more than 10 and 16 g/kg, respectively, and these quantities caused no deaths. Similar amounts of IC/C killed 38 and 60% of the animals, respectively.

Effect of IC/C on ethanol metabolism

The ethanol-acetic acid soluble material extracted from *C. atramentarius* (IC/C) was administered orally, at the estimated LD0 dose of 3·5 g/kg, to groups of mice housed

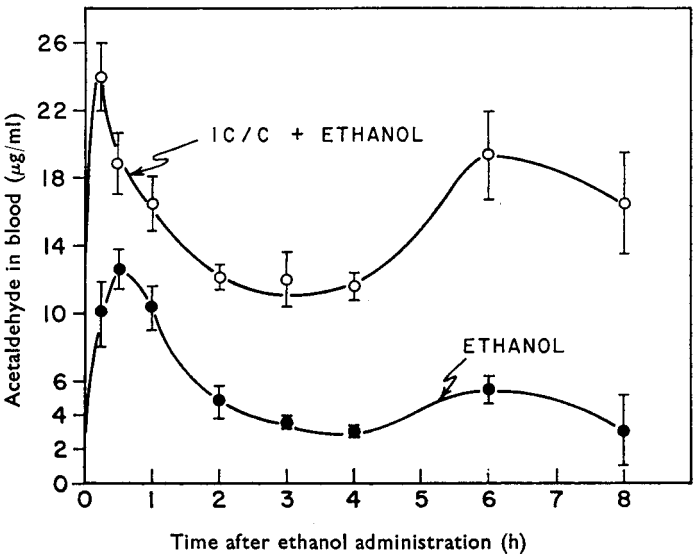

FIG. 1. Acetaldehyde levels in the whole blood of mice administered ethanol alone (—●—●—) and with the ethanol-soluble fraction (IC/C) of *C. atramentarius* (—○—○—). Vertical bars indicate ± one standard error.

5 per cage. Ethanol (6 g/kg) was administered orally 4 h later. Control groups were treated as follows: no treatment; aqueous tragacanth vehicle (2·5 ml/30 g) first, then ethanol 4 h later; IC/C (3·5 g/kg) first, followed by distilled water (0·6 ml/30 g) 4 h later. The animals were decapitated at various times (5 to 10 mice at each time interval) after administration of ethanol or water (controls) and the blood collected in small tubes containing oxalate. The tubes were stoppered and refrigerated until analysed (within 48 h) for acetaldehyde and ethanol by the gas chromatographic procedure of Duritz & Truitt (1964). The results are summarized in Fig. 1 and Table 2.

Table 2. *Blood ethanol concentrations (mg % ± s.e.) in mice treated orally with ethanol, alone and in combination with the ethanol-soluble fraction of* Coprinus atramentarius

Time after ethanol admin. (min)	Treatment		Probability
	Ethanol	IC/C + Ethanol*	
15	356 ± 45 (5)	379 ± 35 (5)	N.S.
30	398 ± 55 (5)	420 ± 93 (5)	N.S.
60	475 ± 81 (10)	443 ± 52 (10)	N.S.
120	547 ± 28 (10)	372 ± 26 (10)	<0·001
180	324 ± 40 (10)	411 ± 32 (8)	N.S.
240	328 ± 68 (5)	550 ± 61 (5)	<0·05
360	179 ± 41 (5)	393 ± 41 (5)	<0·01
480	64 ± 42 (5)	293 ± 67 (5)	<0·02

* IC/C designates the alcohol-soluble fraction of dried, powdered, *Coprinus atramentarius*.

( ) indicate number of animals.

Administration of IC/C resulted in a highly significant increase in the acetaldehyde concentration which was evident 15 min after ethanol was given and persisted throughout the 8 h observation period (Fig. 1). There was no significant difference in the blood ethanol levels in the two groups until at 2 h when the mean concentration in the animals receiving ethanol alone was elevated significantly above the concentration in the animals that were given both IC/C and ethanol. Thereafter, the ethanol concentration in the latter group was higher than in the former at each collection period (Table 2). No acetaldehyde or ethanol was detected in blood from untreated mice given IC/A, IC/B or IC/C only.

DISCUSSION

The present work confirms our earlier observation that *C. atramentarius* potentiates the action of ethanol in mice. We have now shown that the toxicity and the potentiating effect of the ethanol-acetic acid soluble fraction of this mushroom significantly exceeds that of either the whole plant or the ethanol-insoluble residue. The acetaldehyde blood level curves in Fig. 1 suggest that the ethanol-acetic acid soluble fraction inhibits the conversion of acetaldehyde to acetate. The disulfiram-like action of IC/C might be ascribed to the presence of disulfiram in *C. atramentarius*, as claimed by Simandl & Franc (1956), but not confirmed by List & Reith (1960) or by Wier & Taylor (1960). It is possible that other substances are present which inhibit the oxidation of acetaldehyde.

The lower blood ethanol levels at 2 h and the higher levels at 4 to 8 h after ethanol administration in the IC/C + ethanol-treated animals, compared to the levels a these periods in blood from mice given ethanol alone, probably are due to an effect of IC/C on the rate of absorption of ethanol from the gastrointestinal tract, since the rates of disappearance of ethanol from the blood in the two groups of animals (64 and 66 mg %/h, respectively) are essentially similar.

Acknowledgements

We wish to thank Messrs. G. Bélec, W. Skakum and G. Solomonraj for technical assistance; Mr. D. A. Lyon for advice concerning the statistical computations, and Drs. G. S. Wiberg and I. J. McGilveray for helpful criticism of the manuscript.

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