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Potentiated effect of ethanol on Amanita phalloides poisoning

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Interaction of the effects of death cap and ethanol in rats was studied. Ethanol was found to have no protective effect during poisoning by Amanita phalloides. In contrast, it burdened hepatocytes with its own detoxification and made the absorption of the fungal toxins easier due to a changed membrane fluidity. Besides, ethanol was responsible for an increased damage to the cellular membranes by free radicals that originated in its metabolism. The potentiated effects of the two noxae is thus defined.

Our results suggest that the intoxication by A. phalloides parallelled by digestion of a small dose of an alcoholic drink will have a more serious course and worse prognosis.

Key words: Amanita phalloides, ethanol, poisoning

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Byla studována interakce mezi muchomůrkou zelenou a etanolem u laboratorních potkanů. Bylo zjištěno, že etanol nemá protektivní vliv při otravě Amanita phalloides, ale naopak zatěžuje hepatocyt svojí detoxikací, umožňuje lepší vstřebání toxinů houby v důsledku změněné fluidity membrán a také následně při poškození membrán volnými radikály, které se tvoří při jeho metabolismu. Dochází tedy k potenciaci účinků obou nox.

Intoxikace Amanita phalloides spolu i s relativně malou dávkou alkoholického nápoje dle našich výsledků bude mít těžší průběh s horší prognosou.

INTRODUCTION

The interaction of ethanol with coprine (N^5-/hydroxy-cyclopropyl/-L-glutamine), an efficient metabolite in *Coprinus atramentarius*, is well known in mycotoxicology. Coprine inhibits the enzyme aldehyde dehydrogenase (AlDH) that is oxidizing acetaldehyde formed by the ethanol pathway. The accumulation of acetaldehyde results in the antabuse effect accompanied by flush, palpitation, nausea, and vomiting (Hatfield 1975).

Floersheim and Bianchi (1984) and Floersheim (1992) reported a possible protective effect of ethanol during the intoxication by death cap (*Amanita phalloides*). The evidence was brought in experiments using laboratory mice.

The hypothesis concerning the interaction of ethanol in case of *Amanita phalloides* poisoning is different from the above mentioned opinion. Our experiments

were conducted because of our doubts about the above-mentioned conclusions of the Swiss authors.

MATERIALS AND METHODS

The experiments were carried out on female rats (Wistar strain) having an average body weight of 200 g (12-h starvation, water ad libitum, Velaz standard food).

The extract from *Amanita phalloides* was prepared in the following way: an amount of 1 g of dried cap (mature fruit body) was heated to a temperature of 90°C in 20 ml of distilled water and extracted without shaking for 1 h at the room temperature in the dark. The extract was filtered and intraperitoneally applied to the rats at an amount of 1 or 1.5 ml per 200 g body weight. The concentration of toxins in the extract (1.5 ml) represented an LD_{50} dose. Ethanol was applied perorally at a concentration of 33 % by using a gastric tube 30 min before application of *A. phalloides*, the dose being 12.5 ml/kg (Table 1).

Tab. 1 – Experimental scheme (Group I, II – *A. phalloides* and ethanol, III,IV – *A. phalloides*, V – ethanol)

Group of exp. animals	Extract of A. phalloides ml/200g BW	Ethanol ml/kg
I	1,0	12,5
II	1,5	12,5
III	1,0	0
IV	1,5	0
V	0	12,5
Controls	0	0

The lethal effect was estimated a 5 hours after intoxication by *Amanita phalloides*. The liver for histological examination and serum were removed. The enzyme activities (alanine aminotransferase – ALT and aspartate aminotransferase – AST) were determined by spectrophotometric assay (Bio-La test, Lachema Brno). Malondialdehyde (MDA) was determined by spectrophotometric assay the modified Carbonneau's method (reaction with thiobarbituric acid) (Carbonneau et al. 1991). Histological preparations: liver was treated by fixation in the Bouin's liquid and subsequently stained with hematoxylin-eosin.

RESULTS

The mortality rate of the rats resulting from a combined intoxication with the extract of *A. phalloides* and ethanol is shown in Table 2. The death rate observed in the presence of ethanol was twofold as compared to a simple intoxication by the fungal toxins.

Tab. 2 – Mortality rate of rats after intoxication by the extract of *A. phalloides* (III, IV), ethanol (V), combined intoxication with the extract of *A. phalloides* and ethanol (I,II), controls (C).

Group (rat)	Extract A.ph. ml/200gBW	Ethanol ml/kgBW	N	Exitus	%
I	1,0	12,5	10	2	20
II	1,5	12,5	35	35	100
III	1,0	0	10	0	0
IV	1,5	0	35	19	54
V	0	12,5	5	0	0
C	0	0	5	0	0

The activities of aminotransferases (AST, ALT) and the concentration of malondialdehyde were determined only in the animals that survived (control group, group intoxicated only by the extract of *A. phalloides*). The results are summarized in Table 3.

Tab. 3 – Enzyme activities of AST and ALT and the level of MDA in rats after intoxication by the extract of *A. phalloides* (III, IV), ethanol (V), combined intoxication with the extract of *A. phalloides* and ethanol (I, II), controls (C).

Group (rat)	Extract ml/200gBW	Ethanol ml/kg	AST μkat/l	ALT μkat/l	MDA μmol/l
I	1,0	12,5	×	×	×
II	1,5	12,5	×	×	×
III	1,0	0	6,49*	1,64*	4,93
IV	1,5	0	6,65*	1,73*	5,07
V	0	12,5	3,02	0,34	4,39
C	0	0	2,63	0,36	4,19

* statistical significance $p < 0,05$ v.s. controls
× no measured (exitus of rats)

The results (Tab. 3) show a significant rise of the aminotransferase activities in the intoxicated rats by *A. phalloides*. The concentration of the total MDA in blood was not significantly increased in comparison with the control group.

The histological picture of the liver of rats intoxicated with *A. phalloides* and ethanol showed a complet steatosis accompanied by a dilatation of capillary blood vessels and necrotic regions. In the group which received the extract of *A. phalloides*, the steatosis was only slight developed. Areas of destruction were observable, characterized by fusion of the hepatocytes and activation of Kupffer cells.

Clearly, a potentiated effect of *A. phalloides* toxins by ethanol was observed in contrast to the protective effect described by Floersheim et Bianchi (1984).

DISCUSSION

The combination of the effects of ethanol and of the toxins of *A. phalloides* was studied by Floersheim and Bianchi (1984). They used laboratory mice known to be more sensitive to the toxins of *A. phalloides* than rats employed in our study. We have objections against the intraperitoneal application of ethanol used by the Swiss authors. The resulting level of ethanol in the organism is thus increased and, also, the corresponding kinetic and time distribution is different from perorally ethanol administration (Caballeria 1992, Wedel 1991). Metabolism of ethanol by the isoenzymes of alcohol dehydrogenase starts already in the stomach and, accordingly, the ethanol level is decreased before it first pass through liver (Lieber 1990). The ethanol diffuses by the concentration gradient. If ethanol is given perorally, as in our study, its maximal level in blood is reached between 30 and 90 min after administration (Mattila 1990).

Experiments have shown that the toxins of *A. phalloides* – amanitins and phalloidins, accumulate in liver where the amanitines block the function of the enzyme RNA polymerase II(B) and the phalloidins cause polymeration of the protein – actin (Faulstich et Münter 1986, Wieland et Faulstich 1991).

Ethanol is known to affect fluidity and permeability of the cellular membranes and, consequently, increase the penetration of many compounds into the cell (Tarashi et Rubin 1985). The metabolism of ethanol results in a formation of free radicals that cause lipid peroxidation (for instance the cellular membranes) and there are produce aldehydes (e.g. malondialdehyde – MDA, 4-hydroxy nonenal) with toxic effects (Nordmann et al. 1992). The activity of superoxide dismutase (SOD) in the liver and brain, and the liver glutathione peroxidase (GPx) are decreased by ethanol intake (Rouach et al. 1987, Tanner et al. 1986). The levels of glutathione and S-adenosyl-L-methionine are also decreased, which results in a damage of the structure and function of the cellular membranes (Lieber 1990). The cells consist of two antioxidant systems protected against free radical injury. First are the antioxidant enzymes (superoxide dismutase – SOD, glutathione peroxidase – GPx) and second the antioxidant substrates (ascorbic acid, retinol, carotenoids, tocopherols, thiols, bilirubin, etc.) (Halliwell 1991).

The damage to the cellular membranes together with the consequent better permeability for other compounds and the free radicals injury during ethanol metabolism suggest cumulative effects of the two noxae.

The Floersheim (1992) opinion, that SOD capable of scavenger the free radicals during *A. phalloides* poisoning with ethanol does not seem to be likely. Similarly, the possibility that ethanol could interfere with the penetration of the mushroom toxins into the hepatocytes either by blocking the receptors and/or the transport system or via the structural changes of the lipid membrane resulting in an inhibition of entry of the mushroom toxins into the cell is not very convincing.

The Swiss authors linked the clinical data on the higher death rate of children after *A. phalloides* intoxication, as compared to adults, with the possible protective effect of small doses of an alcoholic drink drunk by the adults (Floersheim et Biancki 1984). This explanation seems to be very improbable. We assume that the difference can be explained by a higher amount of the fungus eaten per the total body mass.

Consequently, potentiated effect of ethanol to *A. phalloides* poisoning should be observable by action of free radicals, burdnes the hepatocyte with ethanol detoxification, and a better absorption of the toxins as a result of the changes in cellular membranes due to ethanol and its metabolism. Thus, a phenomenon of potentiation of the toxic effect of *A. phalloides* toxins by ethanol was observed in contrast to the protective effect described by Floersheim et Bianchi (1984). *Amanita phalloides* poisoning with ethanol administration may be worse prognose by our results.

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