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# HEPATO- AND RENAL TOXICITY OF AQUEOUS EXTRACT OF A MUSHROOM, *AMANITA VOLVATA* IN MICE

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***ABSTRACT:*** *Toxic effects of a mushroom, Amanita volvata, were studied in respect to biochemical and histological changes induced in the liver and kidneys of mice. The changes in biochemical parameters characteristically appeared 12 hrs after oral administration of an aqueous extract of the mushroom. The hepatic glycogen decreased markedly to 17% of the control level and a concomitant decrease in blood glucose was also observed. The activity of serum glutamic oxaloacetic transaminase (SGOT) was elevated by 2.5-fold and the level of blood urea nitrogen (BUN) increased by 3-fold, respectively, 12 hrs after administration of the mushroom, their levels being maintained up to 24 hrs. In the urine, protein level was 3-times higher than that of the controls. Histological examination revealed frosted glassy cells in liver and dropsic nephrosis in the kidneys. These results indicate that toxic constituents of A. volvata have the potency to induce hepato- and renal toxicity.*

***Key words:*** *Toxicity of Amanita volvata, Biochemical Parameters, Pathology, Hepatotoxicity, Renal toxicity*

## INTRODUCTION

There are many species of mushrooms in Japan. Though a number of mushroom species are known to cause poisoning in humans, the mechanism of mushroom poisonings has not been fully elucidated. This is partly bacause most of the toxic constituents in poisonous mushrooms have yet to be identified. To obtain useful information for clinical treatment of mushroom poisoning, we have studied the mode of toxic action in mice by aqueous extracts of several poisonous mushrooms and have classified some of them according to the changes of hepatic and renal parameters (Yamaura,

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1981-1986).

The present study deals with the toxic effect of *Amanita volvata* which caused human death before (Food Hygiene Division, 1972).

## MATERIALS AND METHODS

*Amanita volvata* (Japanese name: *Fukurotsurutake*) was collected in Nagano and Shiga Prefectures in Japan. The bodies of mushrooms were cut into small pieces, boiled for 10 min. in 4 volumes of distilled water and filtered. The filtrate was used as an aqueous extract of the mushroom for animal experiments.

Each male mouse (ddY strain 6 weeks old, Nippon SLC Co. Ltd., Shizuoka) was administered orally with the aqueous extract at a dose of 1.5g of fresh mushroom per kg body weight. Control mice received an equal volume of 0.9% saline orally. Food was withdrawn after administration, but the animals had free access to water until they were sacrificed.

Time course changes in biochemical parameters were examined after administration of the mushroom extract. The assay of biochemical parameters in blood and liver of the mice were done 12 hrs after administration of the mushroom extract. The level of blood glucose was determined by the method of Cawley (1959). Serum glutamic-oxaloacetic transaminase (SGOT) was assayed by the method of Reitman and Frankel (1957). The level of blood urea nitrogen (BUN) was determined with a commercially available kit (Wako Pure Chem. Co. Ltd., Osaka). The levels of sodium and potassium in the blood were determined by atomic absorption spectrophotometry (Shimazu Co., Ltd., Model 646). Hepatic glycogen and glutathione were assayed by the method of Morris (1948) and by DTNB[5,5′-dithiobis(2-nitro-benzoic acid)] (Ellman, 1959), respectively.

Urine was collected using a metabolic cage (Sugiyamagen Co. Ltd., Tokyo) for 24 hrs after administration and urinary parameters were measured with Urinary Analyzer (Ames Co. Ltd., U.S.A).

Histological examination was carried out on tissues taken 24 hrs after administration. The liver and kidney were fixed in 10% PBS formalin, embedded in paraffin, and stained with hematoxyline and eosin. Periodic acid-Shiff (PAS) and diastase-PAS reaction were performed.

Data was given as the mean with standard error, and the significane of differences was evaluated by using Student's *t*-test.

## RESULTS

The mice treated with the mushroom extract became moderately prostrate 8 hrs after administration and excreted soft feces.

The level of blood glucose decreased gradually after administration, reaching 60% of the control level 12 hrs after administration (Fig. 1).

SGOT was elevated to 2-times higher than that of the control level 6 hrs after administration and then remained at elevated level throughout the experimental period,

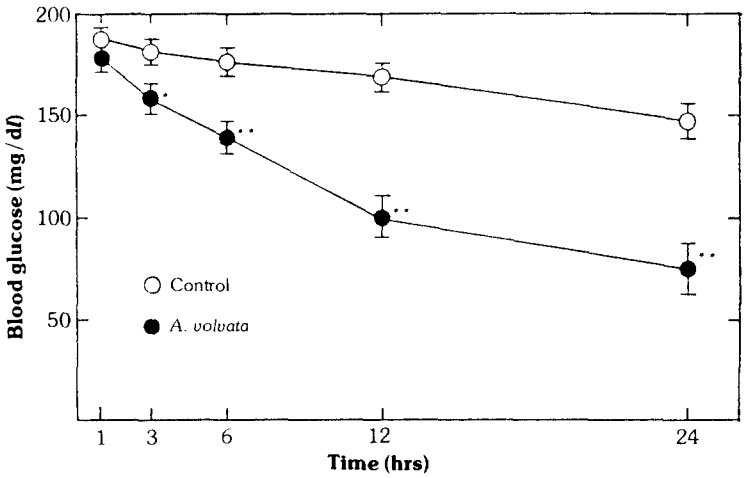



**Fig. 1.** Time course change of blood glucose content after administration of the *A. volvata* extract in mice. Each point represents the mean ± S.E. of five mice. Asterisks indicate the significant differences from control group at $p<0.05$(*) and $p<0.01$ (**).

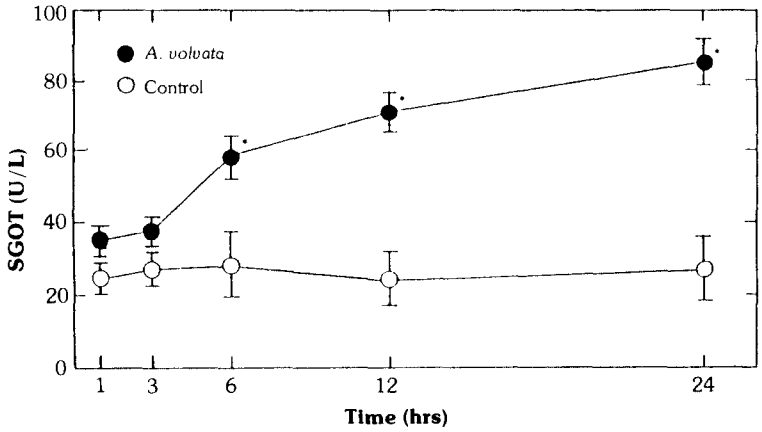



**Fig. 2.** Time course change of SGOT activity after administration of the *A. volvata* extract in mice. Each point represents the mean ± S.E. of five mice. Asterisks indicate the significant difference from control group at $p<0.01$ (*).

showing 3-fold increase over controls at 24 hrs (Fig. 2).

As shown in Fig. 3, a marked increase in BUN level was observed 12 hrs after administration, and the level reached 4-fold of that of the control level 24 hrs after administration.

Effects of the mushroom extract on the changes in components of liver and blood 12 hrs after administration are summarized in Table 1.

The level of hepatic glycogen decreased markedly to 17% of the control level. Significant decrease in the level of liver glutathione (76% of the controls) was also observed. A slight increase was observed in the sodium level in blood. In contrast, the level of potassium in blood decreased significantly.

In the urine, protein level was 3-times higher than that of the control in the mice

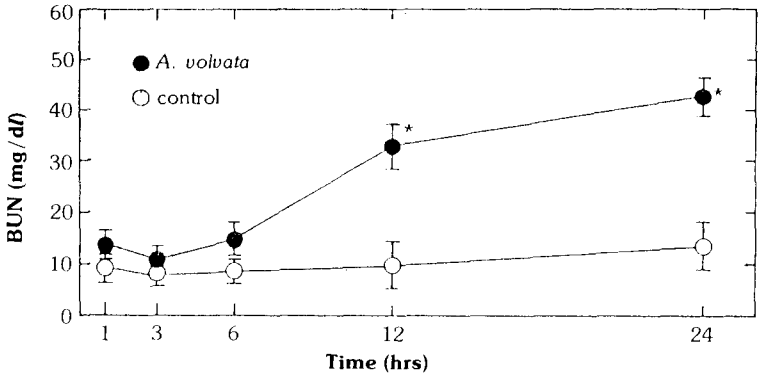



**Fig. 3.** Time course change of BUN content after administration of the *A. volvata* extract in mice. Each point represents the mean ± S.E. of five mice. Asterisks indicate the significant difference from control group at $p<0.01$(*).

**Table 1.** Effect of *A. volvata* extract of components in liver and blood

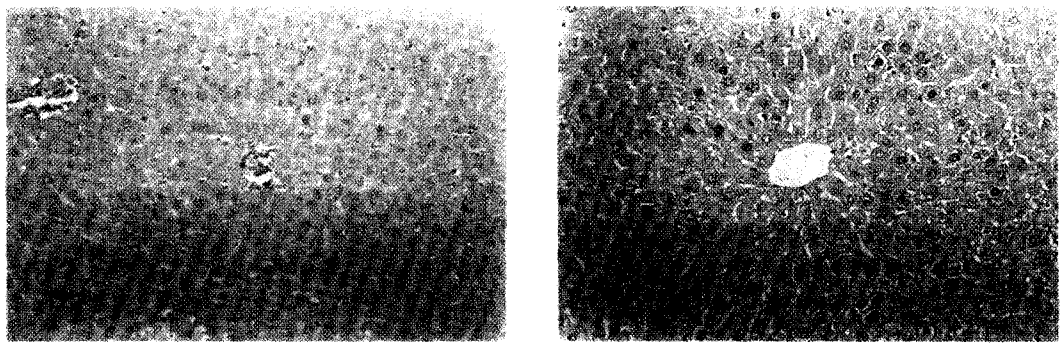
| Parameters | Body wt. (g) | Liver wt. (g/100g body wt) | Liver | | Serum | |
|---|---|---|---|---|---|---|
| | | | Glycogen (mg/g liver) | Glutathione ($\mu$mole/g) | Sodium (meq/l) | Potassium (meq/l) |
| Control | 30.1 ± 0.7 | 4.84 ± 0.23 | 10.3 ± 1.7 | 5.62 ± 0.39 | 142 ± 2 | 8.86 ± 0.39 |
| Treated | 30.2 ± 0.2 | 5.52 ± 0.12* | 1.76 ± 0.15** | 4.29 ± 0.31* | 150 ± 3 | 7.92 ± 0.42** |

Values represent the mean ± S.E. of five mice.
Mice were sacrificed 12 hrs after administration.
Significant differences are with $p<0.05$(*) and $p<0.01$ (**).

**Table 2.** Effect of *A. volvata* extract on urinary parameters

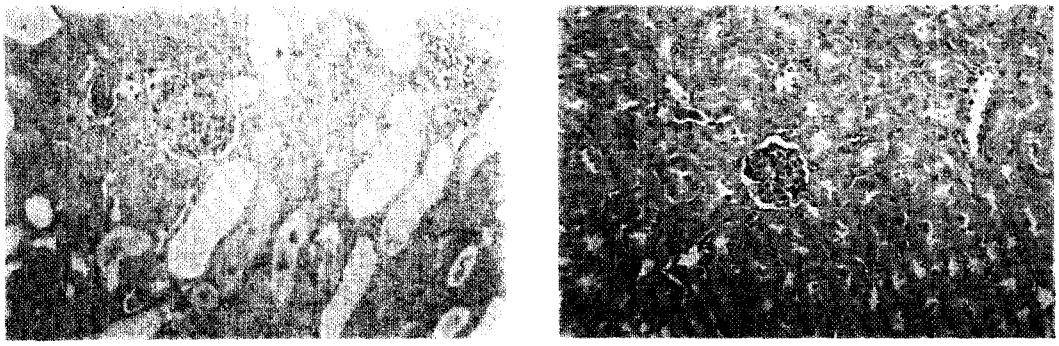
| Parameters | pH | Specific gravity | Protein (mg/dl) | Glucose | Ketone | Bilirubin | Urobilinogen (mg/dl) |
|---|---|---|---|---|---|---|---|
| Control | 8 | 1.015 | ,30 | ND | ND | ND | 0.1 |
| Treated | 9 | 1.010 | 100 | ND | ND | ND | 0.1 |

Urine was collected for 24 hrs after administration.
Values were obtained from pooled samples of five mice.
ND: Not detected.



(a) (b)

**Fig. 4.** Frosted glassy cells and disappearance of PAS-reactive substance in liver were observed 24 hrs after administration. Hematoxylin-eosin stain and × 100. (a) treated, (b) controo, PAS reaction.



(a) (b)

**Fig. 5.** Dropsical nephrosis in kidney was observed 24 hrs after administration. Hematoxylin-eosin stain. ×100. (a) treated, (b) control.

treated with the mushroom (Table 2).

Macroscopically, the liver turned yellowish and its weight increased significantly, and fading decoloration and swelling in the kidneys was observed in the treted mice.

Histological examination demonstrated frosted glassy cells and disappearance of PAS-reactive substances such as hepatic glycogen in the liver, and dropsical nephrosis in the kidneys (Figs. 4 and 5).

## DISCUSSION

Most mushrooms which caused human death are certain species of the genus *Amanita* such as *A. phalloides, A. verna, A. virosa, etc.* (Lincoff and Mitchel, 1977; Ammirati, 1985). *A. volvata* which belong to the genus *Amanita* and are widely distributed in Japan. There have been few reports on the toxicity of the mushroom except for a report of a fatal case which occurred in 1972 (Food Hygiene Division, 1972). The symptoms observed in the poisoning of *A. volvata* were similar to the *Amanita* poisonings, characterized by a sudden onset of abdominal pain, violent vomiting and diarrhea after a latent period of 10-12 hrs after ingestion.

Typical biochemical changes in mice treated with the mushroom occurred in the prolonged phase after administration (12 hrs). The toxic effects of *A. volvata* are less acute than those of other *Amanita* species such as *A. virosa* (6 hrs) (Yamaura, 1981) which caused fatal cases in the *Amanita* poisoning in Japan. These results correspond well to the appearance of symptoms observed in the poisoning in humans.

The decrease in blood glucose level and depletion of hepatic glycogen were also in agreement with the results of histological examination. Though the decrease of the components involved in glucose metabolism might arise from the inhibition of gluconeogenesis, enhancement of glycolysis due to inhibition of $\beta$-oxidation of fatty acids whose mechanism was proposed for the toxic effect of *A. virosa* in our previous studies (Yamaura, 1981; Hashimoto, 1981) or other factors, its significance has not been elucidated in the present study.

The hepatotoxicity of the mushroom has been shown by a marked elevation of SGOT and a significant decrease of biochemical parameters related to hepatic func-

tion. The hepatotoxic potency of the mushroom was lower than the other *Amanita* species as described in our previous studies (Yamaura, 1981, 1986).

Further, the marked increase in the levels of BUN and urinary protein, indicators of kidney function suggested that kidney dysfunction might be provoked by the mushroom. The hepatotoxicity and renal toxicity of *A. volvata* were also confirmed by histological examination.

Another features to be noted are the observations of clinical signs such as soft feces and contrary changes in the levels of sodium and potassium in blood, which might play an important role in the regulation of body fluids. Though the significance of these changes is difficult to evaluate, the changes might be correlated to the appearance of symptoms such as vomiting or diarrhea observed in human poisoning cases.

Concerning the toxic constituents that might be present in *A. volvata*, we could not detect aminitin which is well known as the fatal toxin in the *Amanita* species (Ammirati 1985).

The present studies indicate that *A. volvata* has both acute hepato- and renal toxicity. More studies are necessary, especially on the identification of the toxic constituents in *A. volvata* in order to elucidate the mechanism of the mushroom toxicity in the liver and kidneys.

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