The role of oxidative stress in α-amanitin-induced hepatotoxicity in an experimental mouse model

Zerrin Defne DÜNDAR[1], Mehmet ERGİN[1,*], İbrahim KILINÇ[2], Tamer ÇOLAK[1], Pembe OLTULU[3], Başar CANDER[1]

[1]Department of Emergency Medicine, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey
[2]Department of Biochemistry, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey
[3]Department of Medical Pathology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

Background/aim: This study aimed to evaluate oxidative stress markers of liver tissue in a mouse α-amanitin poisoning model with three different toxin levels.

Materials and methods: The mice were randomly divided into Group 1 (control), Group 2 (0.2 mg/kg), Group 3 (0.6 mg/kg), and Group 4 (1.0 mg/kg). The toxin was injected intraperitoneally and 48 h of follow-up was performed before sacrifice.

Results: Median superoxide dismutase activities of liver tissue in Groups 3 and 4 were significantly higher than in Group 1 (for both, $P = 0.001$). The catalase activity in Group 2 was significantly higher, but in Groups 3 and 4 it was significantly lower than in Group 1 (for all, $P = 0.001$). The glutathione peroxidase activities in Groups 2, 3, and 4 were significantly higher than in Group 1 ($P = 0.006$, $P = 0.001$, and $P = 0.001$, respectively). The malondialdehyde levels of Groups 3 and 4 were significantly higher than Group 1 ($P = 0.015$ and $P = 0.003$, respectively). The catalase activity had significant correlations with total antioxidant status and total oxidant status levels ($r = 0.935$ and $r = -0.789$, respectively; for both, $P < 0.001$).

Conclusion: Our findings support a significant role for increased oxidative stress in α-amanitin-induced hepatotoxicity.

Key words: Amanitin, oxidative stress, mushroom poisoning, biomarker, mouse

1. Introduction

Mushroom poisoning is a serious clinical condition in Turkey and worldwide, commonly occurring in rainy seasons (1,2). Although several mushroom species can be responsible, those of the genus *Amanita* cause the most severe clinical damage. *Amanita* species cause their toxicity via amatoxins (α- and β-amanitin) and phallotoxin (phalloidin). The clinical signs of poisoning, which become obvious 24–72 h after mushroom consumption, especially include severe liver and renal insufficiency (3). Over many years, different treatment modalities, e.g., silibinin, penicillin, cimetidine, and N-acetyl cysteine, aimed at preventing the progressive hepatocyte and renal cell damage in α-amanitin poisoning have been evaluated. Despite those efforts, a clear and effective treatment option has yet to be identified (4–7). Thus, in recent years, particular attention has been given to studies of the pathophysiology of α-amanitin poisoning (8–11).

Oxidative stress is defined as impairment in the balance between reactive oxygen species (ROS) and the antioxidant capacity of living organisms. It has been reported that oxidative stress is the common pathophysiological pathway of most types of intoxications. The role of increased oxidative stress has been demonstrated in experimental organophosphate, paracetamol, and methotrexate models (12–14). Recent in vitro and in vivo studies have suggested that oxidative stress is probably responsible for α-amanitin-induced hepatotoxicity in studies (7,15–18).

To determine the pathways of oxidative stress, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities have been previously evaluated. In recent studies on oxidative stress, total oxidant status (TOS) and total antioxidant status (TAS) have been measured, in addition to the evaluation of the enzymatic activities separately. Measuring TAS and TOS levels provides valuable information regarding the net oxidative stress the organism suffers (19–22). In the available literature, there is no study on α-amanitin poisoning in which TOS and TAS levels are evaluated.

In this experimental study, we aimed to evaluate trends in oxidative stress markers (SOD, CAT, GPx, MDA, TOS, and TAS) in a mouse α-amanitin poisoning model with three different toxin levels.

2. Materials and methods

2.1. Study design
This was an animal model laboratory study using mice. The study protocol was approved by the Ethics Committee for Experimental Animal Studies of the KONÜDAM Application and Research Center of Experimental Medicine (2013/208) and funded by our institutional Scientific Research Projects Coordination Office (Project no. 141218002).

2.2. Animal subjects and preparation
A total of 37 male BALB/c mice, weighing 28–40 g, were used in this experimental study. The mice were fasted for 12 h before the experiment and they were only allowed to drink water during this period.

2.3. Study protocol
The mice were randomly divided into four groups: Group 1 (control group), Group 2 (low-dose poisoning group; 0.2 mg/kg α-amanitin), Group 3 (moderate-dose poisoning group; 0.6 mg/kg α-amanitin), and Group 4 (high-dose poisoning group; 1.0 mg/kg α-amanitin). The control group contained seven mice, and the poisoning groups contained 10 mice each.

After being randomized into their experimental groups, each mouse was weighed with a digital scale. The amount of toxin for each mouse, in accordance with their intoxication group, was calculated and recorded in a table. Our experimental model was designed to have three different levels of poisoning: a low dose, a moderate dose, and a high dose. In the study conducted by Tong et al., the 50% lethal dose value of α-amanitin for mice was 0.6 mg/kg (5). The available literature contains several different mouse α-amanitin poisoning models, in which the preferred dose of toxic α-amanitin ranges from 0.1 to 1.0 mg/kg (9,15–17). In light of these reports, we decided to use 0.2 mg/kg α-amanitin for the low-dose poisoning group, 0.6 mg/kg α-amanitin for the moderate-dose poisoning group, and 1.0 mg/kg α-amanitin for the high-dose poisoning group.

The α-amanitin toxin (Sigma-Aldrich Co., St. Louis, MO, USA) was a white to light yellow powder. One milligram of α-amanitin powder was dissolved in 25 mL of normal saline to obtain a stock solution; 1 mL of this stock solution contained 0.040 mg of α-amanitin.

At hour 0, 1 mL of normal saline was injected into the mice in Group 1 intraperitoneally and the previously calculated dose of α-amanitin was injected into the mice in Groups 2, 3, and 4 intraperitoneally. All mice were then observed for 48 h under the same environmental and feeding conditions.

At hour 48, all mice were sacrificed by exsanguination. Each mouse then underwent laparotomy via a midline incision, and their livers were collected for biochemical and histopathological evaluation.

2.4. Biochemical analysis
The excised liver samples were washed with cool saline several times and stored in cool saline at –80 °C until biochemical analysis. On the day the biochemical assays were performed, the liver tissues were weighed. They were homogenized on ice in 50 mM cool potassium phosphate buffer, pH 7.4, at a ratio of 10% (weight/volume). The liver homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C. Supernatants were collected for biochemical analysis.

SOD, CAT, and GPx activities were detected by using commercial assay kits: the Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA), Catalase Assay Kit (Cayman Chemical Company), and Glutathione Peroxidase Assay Kit (Cayman Chemical Company). MDA levels were measured with thiobarbituric acid (Merck, Darmstadt, Germany) and the reactive substances method described by Okhawa et al. (23). Tissue protein levels were measured with the bicinchoninic acid (BCA) method using a BCA Protein Assay Kit (BioVision, Milpitas, CA, USA).

A Total Oxidant Status Assay Kit (Rel Assay Diagnostics, Gaziantep, Turkey), and Total Antioxidant Status Assay Kit (Rel Assay Diagnostics) were used to determine TOS and TAS levels.

2.4.1. TOS test principle
Oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent (20).

2.4.2. TAS test principle
Antioxidants in the sample reduce dark blue-green colored ABTS radicals to a colorless reduced ABTS form. The change of absorbance at 660 nm is related to the total antioxidant level of the sample. The assay is calibrated with a stable vitamin E analog antioxidant standard solution, traditionally known as Trolox equivalent (19).

A Bio-Rad Xmark microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA) was used for spectrophotometric measurements.

2.5. Histopathological assessment
Liver specimens were fixed with 10% formaldehyde solution for histopathological examination and embedded in paraffin blocks after treatment with a routine xylol–alcohol series. Liver slices with a thickness of 5 mm were stained with hematoxylin and eosin and examined under a light microscope by an independent pathologist blinded to the treatments and controls. Liver slides were evaluated for degeneration (mild, moderate, and marked) and necrosis of hepatocytes (percentage).

2.6. Data analysis
The data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as median (25%–75%). The comparison of biomarkers between experimental groups was performed using Kruskal–Wallis variance analysis and the Mann–Whitney U test with Bonferroni correction. Relationships between biomarkers were assessed using Pearson correlation analysis.

3. Results
At the end of the 48-h observation period, all animals (n = 37) were alive; liver tissues were collected from all animals.

3.1. Biochemical markers
There was no statistically significant difference between Group 2 and Group 1 in terms of median liver tissue SOD activity (P = 0.282). Median liver tissue SOD activity in Group 3 and Group 4 was significantly higher than the median SOD activity in Group 1 (for both, P = 0.001).

The median liver tissue CAT activity in Group 2 was significantly higher than the median CAT activity in Group 1 (P = 0.001). Median liver tissue CAT activity in Groups 3 and 4 was significantly lower than the median CAT activity in Group 1 (for both, P = 0.001).

Median liver tissue GPx activity in Groups 2, 3, and 4 was significantly higher than the median GPx activity in Group 1 (for Group 2, P = 0.006; for Groups 3 and 4, P = 0.001).

There was no statistically significant difference between Group 2 and Group 1 in terms of median liver tissue MDA level (P = 0.064). The median liver tissue MDA levels of Groups 3 and 4 were significantly higher than the median MDA level of Group 1 (for Group 3, P = 0.015; for Group 4, P = 0.003). Median biomarker levels and group comparisons are presented in Table 1.

There was a significant positive correlation between liver CAT activity and liver TAS level, and a significant negative correlation between liver CAT activity and liver TOS level (r = 0.935 and r = –0.789, respectively; for both, P < 0.001). All relationships between biomarkers are presented in Table 2.

Table 1. Comparison of biomarker levels among the groups.

Biomarkers	Group I (Control, n = 7)	Group II (0.2 mg/kg, n = 10)	Group III (0.6 mg/kg, n = 10)	Group IV (1.0 mg/kg, n = 10)	P-value
SOD* U/mg protein	0.261 (0.241–0.269)	0.274 (0.258–0.286)	0.386 (0.382–0.408)	0.389 (0.366–0.404)	<0.001
CAT** U/mg protein	0.154 (0.145–0.158)	0.217 (0.207–0.225)	0.083 (0.065–0.084)	0.056 (0.051–0.062)	<0.001
GSH-Px*** U/mg protein	0.110 (0.101–0.116)	0.129 (0.117–0.132)	0.148 (0.139–0.150)	0.144 (0.129–0.152)	<0.001
MDA† nmol/mg protein	3.68 (3.28–3.99)	4.08 (3.70–4.41)	4.60 (4.20–4.87)	5.01 (4.31–5.36)	0.003
TAS‡ mmol Trolox equiv./g protein	0.484 (0.473–0.488)	0.523 (0.514–0.528)	0.345 (0.328–0.349)	0.217 (0.210–0.231)	<0.001
TOS° μmol H_2O_2 equiv./g protein	4.45 (4.32–4.64)	6.02 (5.46–6.19)	7.92 (7.53–8.19)	10.86 (10.58–10.97)	<0.001

SOD, Superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; TAS, total antioxidant status; TOS, total oxidant status

*SOD: P = 0.282 for I vs. II; P = 0.001 for I vs. III and I vs. IV; P < 0.001 for II vs. III and II vs. IV; P = 0.344 for III vs. IV.

**CAT: P = 0.001 for I vs. II, I vs. III, I vs. IV, and III vs. IV; P < 0.001 for II vs. III and II vs. IV.

***GSH-Px: P = 0.006 for I vs. II; P = 0.001 for I vs. III and I vs. IV; P < 0.001 for II vs. III; P = 0.012 for II vs. IV; P = 0.649 for III vs. IV.

†MDA: P = 0.064 for I vs. II; P = 0.015 for I vs. III; P = 0.003 for I vs. IV; P = 0.049 for II vs. III; P = 0.013 for II vs. IV; P = 0.450 for III vs. IV.

‡TAS: P = 0.001 for I vs. II, I vs. III, and I vs. IV; P < 0.001 for II vs. III, II vs. IV, and III vs. IV.

°TOS: P = 0.001 for I vs. II, I vs. III, and I vs. IV; P < 0.001 for II vs. III, II vs. IV, and III vs. IV.

Table 2. Correlations between biomarker levels in liver tissue.

	r	P-value
TAS vs. TOS	−0.932	<0.001
TAS vs. SOD	−0.851	<0.001
TAS vs. CAT	0.935	<0.001
TAS vs. GSH-Px	−0.645	<0.001
TAS vs. MDA	−0.586	<0.001
TOS vs. SOD	0.814	<0.001
TOS vs. CAT	−0.789	<0.001
TOS vs. GSH-Px	0.702	<0.001
TOS vs. MDA	0.607	<0.001

TAS, Total antioxidant status; TOS, total oxidant status; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

On analyzing the TOS versus TAS graph, we found that the liver TOS level increased progressively with the severity of poisoning. Although the liver TAS level increased in the low-dose poisoning group to compensate for the increased ROS load, it decreased in the moderate- and high-dose poisoning groups. As an overall result, oxidative stress seemed to be increased in moderate- and high-dose α-amanitin poisoning (Figure 1).

3.2. Histopathological assessment
In Group 1, no hepatocyte degeneration or necrosis was found in the liver tissues of any of the animals.

In Group 2, minimal hepatocyte degeneration was observed in the liver tissues of two animals. Hepatocyte necrosis was approximately 5% in eight animals and approximately 10% in two animals.

In Group 3, minimal hepatocyte degeneration was observed in the liver tissues of two animals. Moderate degeneration was observed in three animals and marked degeneration was observed in two animals. Hepatocyte necrosis was approximately 10%–15% in six animals and approximately 15%–25% in four animals.

In Group 4, moderate hepatocyte degeneration was observed in two animals and marked degeneration was observed in eight animals. Hepatocyte necrosis was approximately 30%–40% in three animals, approximately 40%–50% in five animals, and approximately 50%–60% in two animals.

Histopathological findings of the liver slides are presented in Figure 2.

4. Discussion
α-Amanitin belongs to the octapeptide family and is a powerful hepatotoxin. α-Amanitin causes its hepatotoxicity primarily by RNA polymerase II inhibition (24,25). In a study conducted by Zheleva et al. in 2007, it was suggested that increased ROS load and oxidative stress might be involved in α-amanitin-induced hepatotoxicity, in addition to the previously identified pathophysiological mechanism (15). A small number of other studies have supported the relationship between increased oxidative stress and α-amanitin-induced hepatotoxicity (6,7,16–18).

Oxidative stress is a disturbance in the critical balance of ROS production and antioxidant defense systems. If the living organism is unable to increase compensatory antioxidant activity to match the increased ROS load, severe oxidative damage will follow, causing cell death. SOD, CAT, and GPx are the main enzymes of the antioxidant system. SOD catalyzes dismutation of superoxide radicals into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2); CAT is involved in the removal of H_2O_2 by catalytic or peroxidative activity; and GPx catalyzes the reduction of hydroperoxides by reduced glutathione (26,27).

In our study, SOD activity increased as the severity of poisoning increased, although there was no significant difference between the low-dose α-amanitin poisoning and control groups in terms of SOD activity. In a mouse α-amanitin poisoning study, Zheleva et al. reported the same levels of SOD activity in the 0.5 mg/kg α-amanitin and control groups and a higher level in the 1.0 mg/kg α-amanitin group than the controls at 20 h after exposure (15). The SOD activity of human hepatocytes treated with α-amanitin was reportedly significantly higher than controls at 48 h (7). In another mouse study, Marciniak et al. reported that erythrocyte lysate SOD activity increased gradually in 0.1, 0.15, and 0.25 mg/kg α-amanitin poisoning groups at 48 h after exposure (16).
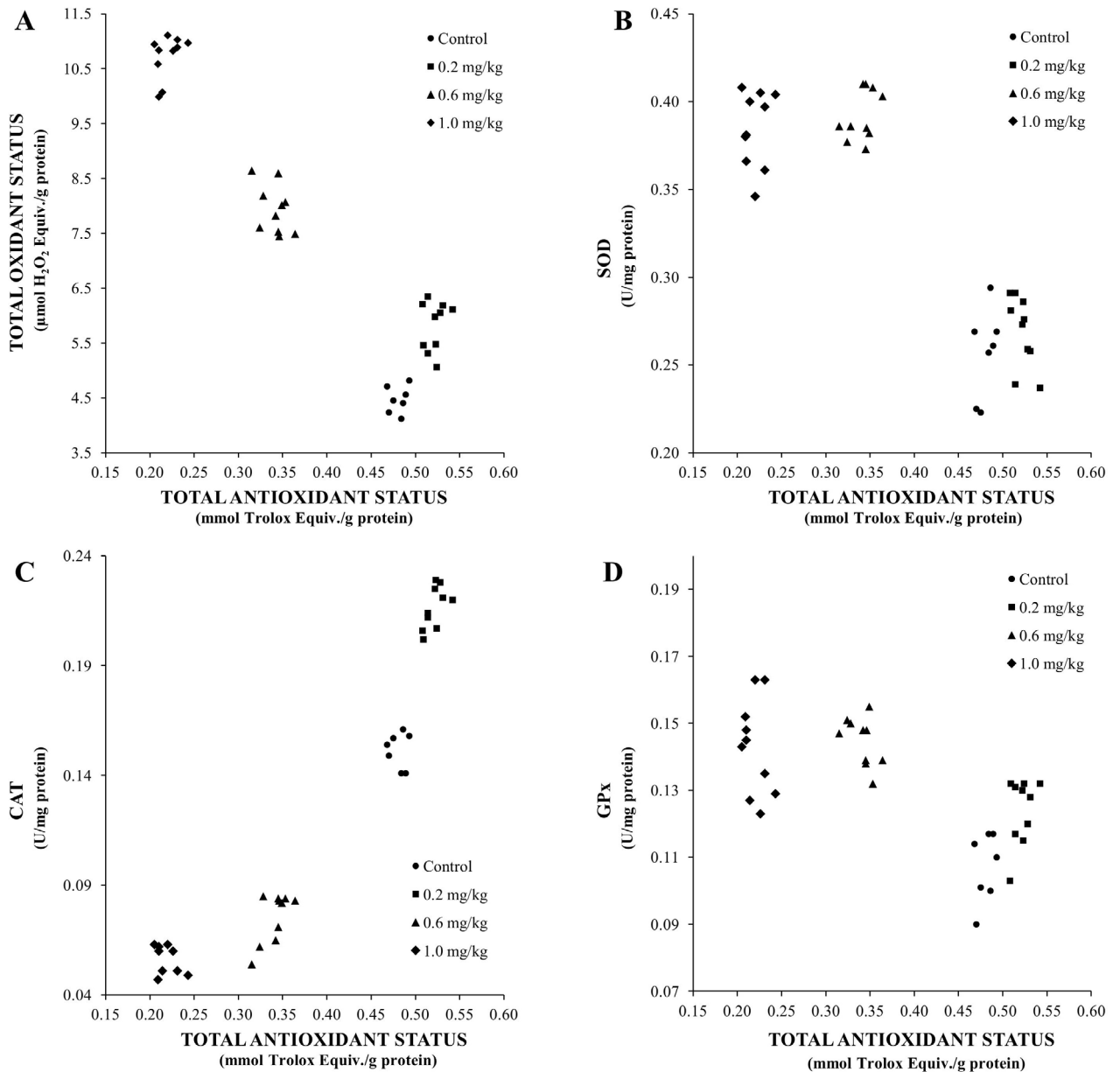
Figure 1. A) Correlation between total antioxidant status and total oxidant status (r = −0.932). B) Correlation between total antioxidant status and superoxide dismutase (SOD) (r = −0.851). C) Correlation between total antioxidant status and catalase (CAT) (r = −0.851). D) Correlation between total antioxidant status and glutathione peroxidase (GPx) (r = −0.645).

In light of those findings, the authors suggested that 20 h was too short for accumulation of α-amanitin in the cells and production of ROS, while 48 h was sufficient (7,15,16). Our results also support an increase in SOD activity in liver tissue treated with a moderate or high dose of α-amanitin after 48 h of exposure. Although our results did not comply with the findings of Marciniak et al. in terms of increased SOD activity in low-dose α-amanitin poisoning, this was probably due to the different SOD activity patterns seen in liver tissue and erythrocytes.

In our study, CAT activity increased in the low-dose α-amanitin poisoning group and decreased in the moderate- and high-dose groups. The CAT activity of human hepatocytes treated with α-amanitin was reportedly detected at significantly lower levels than in controls (7). In a mouse experimental study conducted by Zheleva et al., it was found that the 0.5 and 1.0 mg/kg poisoning groups had significantly lower CAT activity than the control group. However, there was no significant difference between 0.5 and 1.0 mg/kg poisoning groups
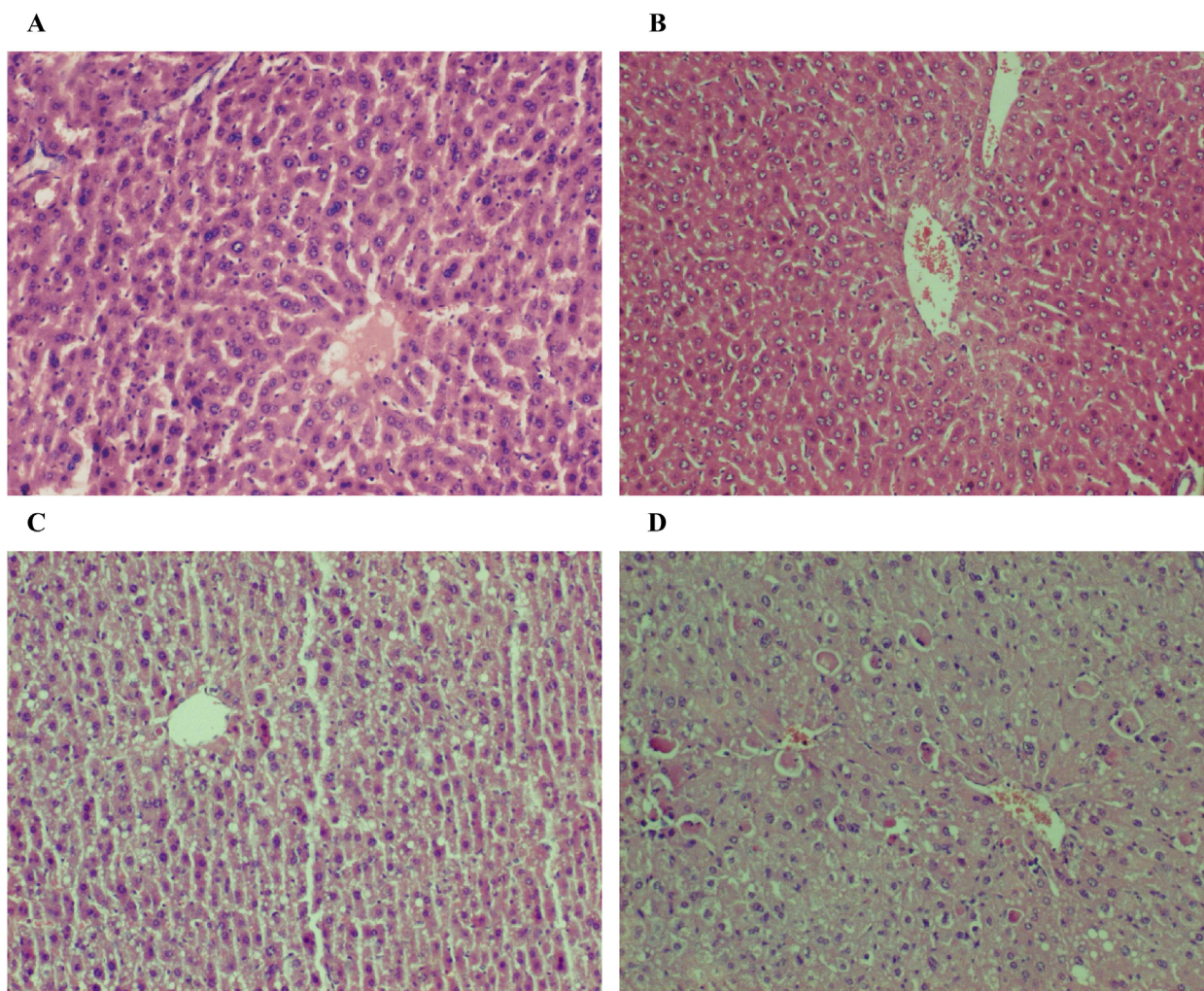
A

B

C

D

Figure 2. Histopathological evaluation of liver tissues with hematoxylin and eosin staining, 100× magnification. A) Group 1 (control), normal liver tissue. B) Group 2 (0.2 mg/kg α-amanitin), lymphocyte infiltration in periportal area and mild hepatocyte degeneration. C) Group 3 (0.6 mg/kg α-amanitin), microvesicular fatty and hydropic hepatocyte degeneration, moderate hepatocyte necrosis. D) Group 4 (1.0 mg/kg α-amanitin), fatty and hydropic hepatocyte degeneration, prominent hepatocyte necrosis.

(15). Marciniak et al. reported that erythrocyte CAT activity decreased in 0.1 and 0.15 mg/kg α-amanitin poisoning groups and increased in the 0.25 mg/kg α-amanitin group when compared with the control (16). In contrast to the findings of Zheleva et al., we found that CAT activity increased in low-dose α-amanitin poisoning in order to overcome the increased ROS load, decreased in moderate-dose poisoning, and then continued to further decrease in high-dose poisoning. Conversely, Zheleva et al. suggested that the direct binding of α-amanitin to CAT might precisely explain decreased CAT activity (15,28). Our results also support the existence of a direct relationship between increased α-amanitin and decreased CAT activity, probably due to CAT inhibition. This suggestion should be further investigated with appropriately designed studies.

In our study, we analyzed liver GPx activity in the setting of α-amanitin poisoning in order to see the whole picture. We found GPx activity in low- and moderate-dose α-amanitin poisoning to be slightly higher than in the controls, but its activity was the same in moderate- and high-dose poisoning groups. In the current literature, investigators have tended not to evaluate hepatocyte GPx activity in α-amanitin poisoning, because they indicated that GPx was responsible for decomposition of H_2O_2 at low levels of H_2O_2 and that CAT was responsible at high levels of H_2O_2 (15,16,29). Conversely, it has been reported that increased ROS load and decreased glutathione levels might play an important role in hepatocyte necrosis induced by toxins, including paracetamol and α-amanitin (26,30). Our results indicated a possible increase in H_2O_2

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production due to increased SOD activity, and GPx was also involved in the antioxidant mechanisms fighting against an increased ROS load. However, GPx did not appear to be sufficiently effective in removing all oxidative stress. In our study, glutathione level was not evaluated in the liver tissues of α-amanitin-poisoned animals. Further investigations should be conducted to elucidate the complete glutathione metabolism involved in α-amanitin poisoning.

In our study, MDA levels tended to increase in the α-amanitin poisoning groups. Although there was no significant difference between MDA levels in the low-dose poisoning group and the control group, the trend in MDA levels suggested that more severe α-amanitin poisoning resulted in an increased ROS load. Reports on MDA levels in the literature are confusing: while some studies reported decreased MDA levels in α-amanitin poisoning, others reported an increase (6,7,15–17). We considered these differences in reported MDA levels to be based on the selected toxin amounts and exposure times used in the studies. Having examined our findings alongside the results of previous studies, we now suggest that ROS load may increase with more severe and prolonged α-amanitin poisoning.

The antioxidant defense mechanism of living organisms contains enzymatic or nonenzymatic antioxidants such as vitamin E, glutathione, and vitamin C (31). In our study, we evaluated the net oxidative stress in liver tissue treated with different doses of α-amanitin by measuring TOS and TAS levels in addition to the antioxidant enzymes SOD, CAT, and GPx. To the best of our knowledge, ours was the first experimental mouse model of α-amanitin poisoning to evaluate TOS and TAS. We were therefore unable to compare our results with those of previous studies. However, in an experimental rat study of paracetamol-induced hepatotoxicity, rats treated with paracetamol were reported to have higher TOS levels and lower TAS levels than the controls (14). Additionally, TOS levels have been shown to increase in rats treated with methotrexate, while TAS levels remained unchanged (13). When we evaluated TOS and TAS levels together, we found that TOS levels tended to increase through α-amanitin poisoning groups, TAS levels increased in low-dose α-amanitin poisoning to compensate for the increased ROS load, and TAS levels decreased with moderate- and high-dose α-amanitin poisoning. Additionally, we evaluated the relationship between TAS level and three antioxidant enzymes. In the liver tissue of mice treated with α-amanitin, TAS level was directly related to CAT activity (r = 0.935). In low dose α-amanitin poisoning, CAT activity increased slightly as a result of increased TOS levels in order to improve antioxidant capacity and TAS. However, in higher dose α-amanitin poisoning, CAT activity and TAS levels decreased dramatically, probably due to the direct inhibition of CAT by α-amanitin.

In conclusion, our findings support a significant role for increased oxidative stress in α-amanitin-induced hepatotoxicity. To achieve effective treatment modalities for α-amanitin poisoning, investigators might direct their efforts towards discovering how antioxidant capacity can be strengthened. In particular, identifying the pathophysiological mechanism responsible for the direct relationships among CAT, α-amanitin, and TAS levels may be very helpful for further treatment investigations.

This study is an experimental mouse model of α-amanitin poisoning. An animal model may not represent the same antioxidant response seen in human beings. Also, the pharmacokinetics and pharmacodynamics of orally ingested α-amanitin may differ from α-amanitin injected intraperitoneally.

Although we evaluated oxidant and antioxidant systems using three different doses of α-amanitin, we did not evaluate the changes in those biomarkers over time after exposure. Changes in oxidative biomarkers over time should be investigated with further α-amanitin poisoning models in order to develop precise treatment options.

References

1. Schenk-Jaeger KM, Rauber-Lüthy C, Bodmer M, Kupferschmidt H, Kullak-Ublick GA, Ceschi A. Mushroom poisoning: a study on circumstances of exposure and patterns of toxicity. Eur J Intern Med 2012; 23: e85-91.

2. Koyuncu M, Öztürk D, Benli AR, Altınbilek E, Şahin H, Serin M, İkizceli İ. Mushroom poisoning in a metropolitan hospital. Journal of Academic Emergency Medicine 2014; 13: 162-165.

3. Shih RD. Plants, mushrooms, and herbal medications. In: Marx JA, Hockberger RS, Walls RM, editors. Rosen's Emergency Medicine: Concepts and Clinical Practice. 8th ed. Philadelphia, PA: Saunders; 2014. pp. 2066-2075.

4. Ward J, Kapadia K, Brush E, Salhanick SD. Amatoxin poisoning: case reports and review of current therapies. J Emerg Med 2013; 44: 116-121.

5. Tong TC, Hernandez M, Richardson WH 3rd, Betten DP, Favata M, Riffenburgh RH, Clark RF, Tanen DA. Comparative treatment of alpha-amanitin poisoning with N-acetylcysteine, benzylpenicillin, cimetidine, thioctic acid, and silybin in a murine model. Ann Emerg Med 2007; 50: 282-288.

6. Magdalan J, Ostrowska A, Piotrowska A, Gomułkiewicz A, Podhorska-Okołów M, Patrzałek D, Szelag A, Dziegiel P. Benzylpenicillin, acetylcysteine and silibinin as antidotes in human hepatocytes intoxicated with alpha-amanitin. Exp Toxicol Pathol 2010; 62: 367-373.

7. Magdalan J, Piotrowska A, Gomułkiewicz A, Sozański T, Szeląg A, Dziegięl P. Influence of commonly used clinical antidotes on antioxidant systems in human hepatocyte culture intoxicated with alpha-amanitin. Hum Exp Toxicol 2011; 30: 38-43.

8. Khunnala A, Narongchai S, Butkrachang S, Leelarungrayub D, Naraongchai P. Anti-oxidative stress activities of silibinin on alpha-amanitin in vitro. Thai Journal of Toxicology 2009; 24: 106-112.

9. Zhao J, Cao M, Zhang J, Sun Q, Chen Q, Yang ZR. Pathological effects of the mushroom toxin alpha-amanitin on BALB/c mice. Peptides 2006; 27: 3047-3052.

10. May JP, Fournier P, Patrick BO, Perrin DM. Synthesis, characterisation, and in vitro evaluation of Pro2-Ile3-S-deoxo-amaninamide and Pro2-D-allo-Ile3-S-deoxo-amaninamide: implications for structure-activity relationships in amanitin conformation and toxicity. Chemistry 2008; 14: 3410-3417.

11. Gundula S, Wells LD, Milliano MT, Talkad V, Luxon BA, Neuschwander-Tetri BA. The hepatocellular bile acid transporter Ntcp facilitates uptake of the lethal mushroom toxin alpha-amanitin. Arch Toxicol 2004; 78: 68-73.

12. Ranjbar A, Solhi H, Mashayekhi FJ, Susanabdi A, Rezaie A, Abdollahi M. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. Environ Toxicol Pharmacol 2005; 20: 88-91.

13. Akbulut S, Elbe H, Eris C, Dogan Z, Toprak G, Otan E, Erdemli E, Turkoz Y. Cytoprotective effects of amifostine, ascorbic acid and N-acetylcysteine against methotrexate-induced hepatotoxicity in rats. World J Gastroenterol 2014; 20: 10158-10165.

14. Saritas A, Kandis H, Baltaci D, Yildirim U, Kaya H, Karakus A, Colakoglu S, Memisogullari R, Kara IH. N-Acetyl cysteine and erdosteine treatment in acetaminophen-induced liver damage. Toxicol Ind Health 2012; 30: 670-678.

15. Zheleva A, Tolekova A, Zhelev M, Uzunova V, Platikanova M, Gadzheva V. Free radical reactions might contribute to severe alpha amanitin hepatotoxicity--a hypothesis. Med Hypotheses 2007; 69: 361-367.

16. Marciniak B, Lopaczyńska D, Kowalczyk E, Skośkiewicz J, Witczak M, Majczyk M, Grabowicz W, Ferenc T. Evaluation of micronuclei in mice bone marrow and antioxidant systems in erythrocytes exposed to α-amanitin. Toxicon 2013; 63: 147-153.

17. Nikolova G, Karamalakova Y, Hadjibojeva P, Georgiev Ts, Tolekova A, Gadjeva V, Zheleva A. Severe mushroom toxin alpha amanitin causes generation of reactive oxygen species in liver tissues of mice – a comparative study by two different instrumental methods. Trakia Journal of Sciences 2010; 8: 149-154.

18. Wu X, Zeng J, Hu J, Liao Q, Zhou R, Zhang P, Chen Z. Hepatoprotective effects of aqueous extract from Lingzhi or Reishi medicinal mushroom Ganoderma lucidum (higher basidiomycetes) on α-amanitin-induced liver injury in mice. Int J Med Mushrooms 2013; 15: 383-391.

19. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112-119.

20. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-1111.

21. Cighetti G, Bamonti F, Aman CS, Gregori D, De Giuseppe R, Novembrino C, de Liso F, Maiavacca R, Paroni R. Oxidative status in different settings and with different methodological approaches compared by Receiver Operating Characteristic curve analysis. Clin Biochem 2015; 48: 73-78.

22. Karkucak M, Capkin E, Alver A, Akyuz A, Kiris A, Ak E, Topbas M, Tosun M. The effect of anti-TNF agent on oxidation status in patients with ankylosing spondylitis. Clin Rheumatol 2010; 29: 303-307.

23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.

24. Kaplan CD, Larsson KM, Kornberg RD. The RNA polymerase II trigger loop functions in substrate selection and is directly targeted by alpha-amanitin. Mol Cell 2008; 30: 547-556.

25. Chafin DR, Guo H, Price DH. Action of alpha-amanitin during pyrophosphorolysis and elongation by RNA polymerase II. J Biol Chem 1995; 270: 19114-19119.

26. Han D, Hanawa N, Saberi B, Kaplowitz N. Mechanisms of liver injury. III. Role of glutathione redox status in liver injury. Am J Physiol Gastrointest Liver Physiol 2006; 291: G1-7.

27. Klaunig JE, Wang Z, Pu X, Zhou S. Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicol Appl Pharmacol 2011; 254: 86-99.

28. Zheleva A, Tolekova A, Zhelev M, Zhelev M, Dobreva Z, Halacheva K, Popova S. In vivo antioxidant and prooxidant properties of Amanita phalloides mushroom toxins. Trakia Journal of Sciences 2005; 3: 34-38.

29. Carpena X, Wiseman B, Deemagarn T, Singh R, Switala J, Ivancich A, Fita I, Loewen PC. A molecular switch and electronic circuit modulate catalase activity in catalase-peroxidases. EMBO Rep 2005; 6: 1156-1162.

30. Hentze H, Gantner F, Kolb SA, Wendel A. Depletion of hepatic glutathione prevents death receptor-dependent apoptotic and necrotic liver injury in mice. Am J Pathol 2000; 156: 2045-2056.

31. Saeidnia S, Abdollahi M. Toxicological and pharmacological concerns on oxidative stress and related diseases. Toxicol Appl Pharmacol 2013; 273: 442-455.