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Alpha-Amanitin Poisoning, Nephrotoxicity and Oxidative Stress: An Experimental Mouse Model

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Background: Alpha-amanitin (α-AMA) plays a major role in *Amanita phalloides* poisoning, showing toxic effects on multi-organs, particularly on the liver and kidneys. Studies have shown a relationship between α-AMA-related injuries and reactive oxygen species.
Objectives: We aimed to investigate whether there is renal injury and its relationship with oxidative stress after intraperitoneal injection of α-AMA in mice experimental poisoning models.
Materials and Methods: There were 37 male BALB/c laboratory mice treated with α-AMA, according to the study groups: control group (n = 7); low dose (0.2 mg/kg) (n = 10); moderate dose (0.6 mg/kg) (n = 10), and high dose (1 mg/kg) (n = 10). The sample size was detected according to the ethical committee's decision as well as similar studies in the literature. After a 48-hour follow-up period, all the subjects were sacrificed for pathological and biochemical assays. The study was held in Turkey.
Results: α-AMA poisoning in mice results in inflammatory changes and necrosis in renal structures. There were statistically significant differences between the study groups regarding measured levels of catalase, superoxide dismutase, glutathione peroxidase, total antioxidant status (TAS), total oxidant status (TOS) and malonyl dialdehyde in renal homogenates of mice ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.001$, respectively). The TOS and TAS measurements helped to eliminate cumbersome analysis of diverse oxidant and antioxidant molecules. The TOS levels in renal homogenate of mice were significantly higher in all the intoxication groups compared to the control group (5.73, 7.02, 7.77, and 9.65 mmol trolox eq/g protein and $P = 0.002$, $P = 0.001$, and $P = 0.001$, respectively). The TAS levels in moderate and high-dose groups were significantly lower than all the other groups treated with α-AMA (0.130, 0.152, 0.065, and 0.087 mmol trolox eq/g protein and $P = 0.031$, $P = 0.001$, and $P = 0.001$, respectively).
Conclusions: Our results indicated that α-AMA poisoning in mice led to inflammatory changes and necrosis in renal structures. Biochemical analysis showed a shift in the oxidative/anti-oxidative balance towards the oxidative status.

Keywords: Alpha-Amanitin; Oxidative Stress; Mycotoxins

1. Background

Mushroom poisoning is a common environmental medical emergency. Turkey has a rich macro-fungal flora due to optimal ecological conditions. Although only 50 - 100 of about 5000 known mushroom species are toxic, it is difficult for the untrained and unqualified people to differentiate between the toxic and the nontoxic ones. In Turkey, there has been an increase in mushroom poisoning in recent years, especially during spring and fall. A wide clinical spectrum of symptoms can be observed, ranging from simple gastroenteritis to life-threatening hepatorenal failure. *Amanita phalloides* is responsible for 95% of mushroom poisoning (1-3). *Amanita* species cause intoxication signs via amatoxins (α and β-amanitin) and phallotoxins (phalloidin) (4, 5). The diagnosis of amatoxin intoxication can be challenging due to delayed onset of the symptoms. In addition, there is controversial data on many treatment options (repetitive doses of activated charcoal, N-acetylcysteine, penicillin, cimetidine, silymarin and plasmapheresis) (4, 6-10).

α-Amanitin (α-AMA) plays a major role in *A. phalloides* poisoning, showing toxic effects on multi-organs, particularly the liver and kidneys. The main molecular mechanism responsible for intoxication signs was found to be the inhibition of ribonucleic acid (RNA) polymerase II in eukaryotic cells. α-AMA has also been claimed to have additional effects on other cellular structures. Studies have shown a relationship between α-AMA and reactive oxygen species (ROS) (11-16).

ROS, including superoxide, hydrogen peroxide (H_2O_2), hydroxyl, and nitric oxide are induced by several endog-

enous and exogenous sources. Oxidative stress occurs when ROS overcome the antioxidant molecules and the DNA repair mechanisms in the cell, resulting in oxidative damage. Studies investigating oxidative stress have evaluated ROS products such as thiobarbituric acid reactive substance (TBARS) (malondialdehyde (MDA) and other aldehydes) and activities of enzymes taking part in antioxidant systems such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxide (GSH-Px) (17-19). In recent years, the evaluation of each of the enzymatic pathway separately as well as the measurements of total antioxidant state (TAS) and total oxidant state (TOS) have allowed the assessment of antioxidant capacity and the increased ROS load together in living things (20, 21).

Studies on markers of oxidative stress using mushroom poisoning models have been limited to hepatocytes and erythrocytes. In those in vivo and in vitro experimental studies, the activation of antioxidant enzyme defense system in hepatocytes and erythrocytes upon exposure to α-AMA has been a supporting evidence for the formation of ROS (22-24).

2. Objectives

In our study, using an in vivo mouse poisoning model arranged via intraperitoneal (IP) injection of α-AMA, we aimed to investigate whether there is renal injury and its relationship with oxidative stress. This is the first study regarding the investigation of nephrotoxicity in an in vivo α-AMA intoxication model.

3. Materials and Methods

This experimental study was held in July 2014 in Konya, Turkey.

3.1. Overview to the Experimental Animal Groups

Thirty-seven male BALB/c laboratory mice were used in this study. All the animals were kept under standard controlled conditions (12-hour light/12-hour dark cycles at a constant temperature of 18-23°C and humidity of 40% - 70%). They were allowed free access to standard laboratory chow and tap water. The experimental animals ethical committee of the Necmettin Erbakan university Kombassan experimental medicine research and application center approved all the experimental procedures (25.12.2013; decision number: 2013-28).

The sample size was calculated with a power of 0.95 and type I error of 0.05; so that we found 5 mice to each group. However, we examined 10 mice for the intoxicated groups to increase the power of the study. The mice were randomly categorized into four groups: control (n = 7) and intoxication groups exposed to α-AMA at three different doses (n = 10). Randomization was carried out using random number generator in Excel package software.

The experiments were carried out on the following groups: control group (group I); low-dose (0.2 mg/kg) α-AMA group (group II); moderate-dose (0.6 mg/kg) α-AMA group (group III); high-dose (1 mg/kg) α-AMA group (group IV).

α-AMA toxin (Prod. No. A2263, Sigma-Aldrich Co., St Louis, USA) was used to produce the mushroom poisoning model. Using 1 mg of α-AMA powder, the stock toxin solution was prepared by filling it up to 25 mL with 0.9% NaCl; 1 mL of the stock solution contained 0.04 mg α-AMA toxin. After the determination of the experiment groups, each subject was weighed with a delicate balance and the amount of toxin to be injected to each group was determined.

3.2. Induction of Intoxication and Follow-up

At the beginning of the experiment, toxin was applied to each subject via IP injection at doses determined before. According to this, group I was injected with 1 mL of 0.9% NaCl IP; for groups II, III and IV, the calculated amounts of toxin were injected IP by reaching the total volume to 1 mL with 0.9% NaCl. After the IP injections, the subjects were monitored during the following 48 hours in their own environment. The subjects were allowed to eat and drink.

3.3. Sacrificing and Excision of Biomaterials to be Examined

After the 48-hour follow-up period, all the subjects were sacrificed via intracardiac blood sampling. Following sacrificing, median-line incision was made in each subject. Renal tissues of each subject were removed for biochemical and pathologic examinations.

3.4. Evaluation of Materials by Enzyme Analysis

The obtained renal tissue samples were rinsed with cold saline solution. They were stored at -80°C after adding cold saline again until the day of biochemical analysis. On the day of analysis, after weighing the tissues, 50 mM, pH 7.4 cold potassium phosphate buffer was added in 10% (weight/volume) ratio and the tissues were surrounded with ice and were homogenized. The homogenate was centrifuged at 10000 g at 4°C for 20 minutes. The supernatant was separated for biochemical analyses. The calibration authorities of medical devices agency considered all the devices used during our experiment. The calibration is valid up to first of October 2015.

For biomarker measurements, Superoxide Dismutase Assay Kit (Cayman chemical company, item No. 706002, Michigan, USA), Catalase assay kit (Cayman chemical company, item No. 707002, Michigan, USA), Glutathione Peroxidase assay kit (Cayman chemical company, item No. 703102, Michigan, USA), total oxidant status assay kit (Rel Assay Diagnostics, prod. code RL0024, Gaziantep, Turkey), total antioxidant status assay kit (Rel Assay Diagnostics, prod. code RL0017, Gaziantep, Turkey) and commercial assay kits were used. The MDA levels were measured using thiobarbituric acid reactive substance (TBARS) (Merck, Darmstadt, Germany), defined by Ohkawa et al. (25). Tissue protein levels were measured by Bicinchoninic acid

(BCA) method using commercial BCA Protein assay kit (BioVision, IL, USA). BioRad xMark microplate spectrophotometer (Bio-Rad Laboratories, CA, USA) was used for spectrophotometric measurements. The accuracy of spectrophotometer was tested by potassium dichromate solution at certain concentrations dissolved in sulfuric acid.

3.5. Pathological Evaluation of the Materials

Renal specimens were fixed with 10% formaldehyde solution for histopathological examination. The specimens fixed were gradually and progressively dehydrated in alcohols for eight hours in a tissue processing machine. Thereafter, they were embedded in paraffin blocks. Thin (5 μm) renal slices were cut using microtome and were placed on slides. The slides were stained with hematoxylin and eosin (H & E) and examined under a light microscope (Olympus BX 51, NY, USA) by an independent pathologist blind to the treatments and controls. The specimens were evaluated for the presence of α-AMA-related nephrotoxicity, lymphocytic infiltration, loss of brush border, hydropic degeneration, necrosis and thyroidization.

3.6. Statistical Analysis

All the obtained data was analyzed using SPSS software (version 16.0, SPSS Inc., Chicago, IL). Normality assessment was performed using Kolmogorov-Smirnov and Shapiro-Wilk Tests. The data did not fit into the normal distribution. The data was expressed as median (25% - 75%). Differences in biochemical marker levels between all the groups were assessed with Kruskal Wallis test. For markers showing statistically significant differences and comparisons between the two groups, the data were analyzed using Mann-Whitney U test with Bonferroni correction.

4. Results

4.1. Macroscopic Appearance

At the end of the 48-hour follow-up period, all the 37 subjects were alive. After sacrificing and incision, renal structures were compared morphologically. As for comparison of macroscopic renal appearances between the control group and the high-dose α-AMA group, marked hypertrophy was a relevant finding.

4.2. Biochemical Markers

There were statistically significant differences between the study groups regarding the measured levels of CAT, SOD, GSH-Px, TAS, TOS and MDA in renal homogenates of mice, which were prepared according to the study protocol ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.001$, respectively). Median values of biochemical markers in the groups as well as the results of comparisons between two groups are shown in Tables 1 and 2.

According to our findings, CAT activity in renal homogenate of mice was significantly reduced in low-dose and high-dose α-AMA groups compared to the control group (0.155, 0.126, 0.155, and 0.124 U/mg protein and $P = 0.006$, $P = 0.493$, and $P = 0.005$, respectively). However, there was no difference between the low-dose and high-dose groups ($P = 0.226$). As for the moderate-dose group, there was no difference between this group and the control group ($P = 0.493$; Table 2).

SOD activity in renal homogenate of mice was reduced in the low-dose α-AMA group compared with the control group (0.221, 0.156, 0.356, and 0.339 U/mg protein and $P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively). In the moderate-dose group, SOD activity was higher than both control and low-dose groups ($P = 0.001$ and $P < 0.01$, respectively). In the high-dose group, SOD activity was significantly higher than the other groups ($P = 0.001$, $P < 0.001$, and $P = 0.001$, respectively) (Table 2).

GSH-Px activity in renal homogenate of mice showed a statistically insignificant increase in the low-dose α-AMA group compared with the control group (0.064, 0.069, 0.114, and 0.084 U/mg protein and $P = 0.922$, $P = 0.001$, and $P = 0.008$, respectively). In the moderate-dose group, GSH-Px activity was significantly higher than both control and low-dose groups ($P = 0.001$ and $P < 0.001$, respectively). As for the high-dose group, GSH-Px activity was significantly lower than the moderate-dose group ($P = 0.001$; Table 2).

The TAS level in renal homogenate of mice showed a statistically significantly increase in the low-dose α-AMA group compared with the control group (0.130, 0.152, 0.065, and 0.087 mmol Trolox eq/g protein and $P = 0.031$, $P = 0.001$, and $P = 0.001$, respectively). However, in the moderate-dose group, the TAS levels were significantly lower than both control and low-dose groups ($P = 0.001$ and $P < 0.001$, respectively). In addition, the TAS level in the high-dose group was significantly lower than all the other groups treated with α-AMA ($P = 0.001$ and $P < 0.001$, respectively) (Table 2 ; Figure 1).

The TOS levels in renal homogenate of mice were significantly higher in all the intoxication groups compared to the control group (5.73, 7.02, 7.77, and 9.65 mmol trolox eq/g protein; $P = 0.002$, $P = 0.001$, and $P = 0.001$, respectively). As the level of toxin increased, the TOS levels also were found to become higher, showing significant differences between the incremental intoxication groups ($P = 0.034$, $P = 0.001$, and $P = 0.041$, respectively) (Table 2 ; Figure 1).

The examination of the graphic showing TAS and TOS levels in renal homogenate of mice showed that the TOS levels increased and the TAS levels decreased remarkably in toxic exposures (≥ 0.6 mg/kg) (Figure 1). This finding indicated the disruption of oxidative/antioxidative balance in the kidney.

The MDA products in renal homogenates of mice were significantly higher in all the intoxication groups compared to the control group ($P = 0.002$, $P = 0.001$, and $P = 0.002$, respectively). There was no difference between the intoxication groups in this regard ($P = 0.026$, $P = 0.151$, and $P = 0.705$, respectively) (Table 2).

Table 1. The Median Values of Biochemical Markers and Comparison of the Study Groups in Mice Renal Homogenate [a,b]

	Group I (Control; n = 7)	Group II (0.2 mg/kg; n = 10)	Group III (0.6 mg/kg; n = 10)	Group IV (1 mg/kg; n = 10)	P Value
CAT U/mg protein	0.155 (0.149 - 0.156)	0.126 (0.118 - 0.135)	0.155 (0.146 - 0.163)	0.124 (0.114 - 0127)	< 0.001
SOD U/mg protein	0.221 (0.199 - 0.232)	0.156 (0.153 - 0.177)	0.356 (0.323 - 0.362)	0.399 (0.391 - 0.403)	< 0.001
GSH-Px U/mg protein	0.064 (0.063 - 0.076)	0.069 (0.066 - 0.071)	0.114 (0.110 - 0.116)	0.084 (0.077 - 0.094)	< 0.001
TAS Mmol trolox eq/g protein	0.130 (0.128 - 0.135)	0.152 (0.134 - 0.156)	0.065 (0.058 - 0.079)	0.087 (0.075 - 0.092)	< 0.001
TOS Mmol trolox eq/g protein	5.73 (4.82 - 6.08)	7.02 (6.51 - 7.65)	7.77 (7.06 - 8.92)	9.65 (7.87 - 9.81)	< 0.001
MDA U/mg protein	5.13 (4.36 - 5.55)	6.79 (6.50 - 6.94)	7.34 (6.66 - 7.56)	7.01 (6.51 - 7.68)	0.001

[a] Data is expressed as median (25% - 75%).
[b] Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malonyl dialdehyde; SOD, superoxide dismutase; TAS, Total antioxidant status; TOS, total oxidant status.

Table 2. The Paired Comparison of Biomarkers in Mice Renal Homogenate Between the Study Groups [a]

	P Values of Pairwise Comparisons					
	I vs. II	I vs. III	I vs. IV	II vs. III	II vs. IV	III vs. IV
CAT U/mg protein	0.006	0.493	0.005	< 0.001	0.226	< 0.001
SOD U/mg protein	0.001	0.001	0.001	< 0.001	< 0.001	0.001
GSH-Px U/mg protein	0.922	0.001	0.008	< 0.001	< 0.001	0.001
TAS mmol trolox eq/g protein	0.031	0.001	0.001	<0.001	< 0.001	0.006
TOS mmol trolox eq/g protein	0.002	0.001	0.001	0.034	0.001	0.041
MDA U/mg protein	0.002	0.001	0.002	0.026	0.151	0.705

[a] Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; MDA: malonyl dialdehyde SOD, superoxide dismutase; TAS, Total antioxidant status; TOS, total oxidant status.

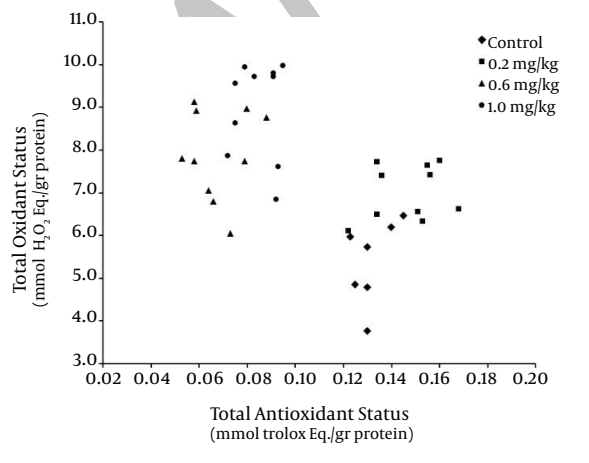


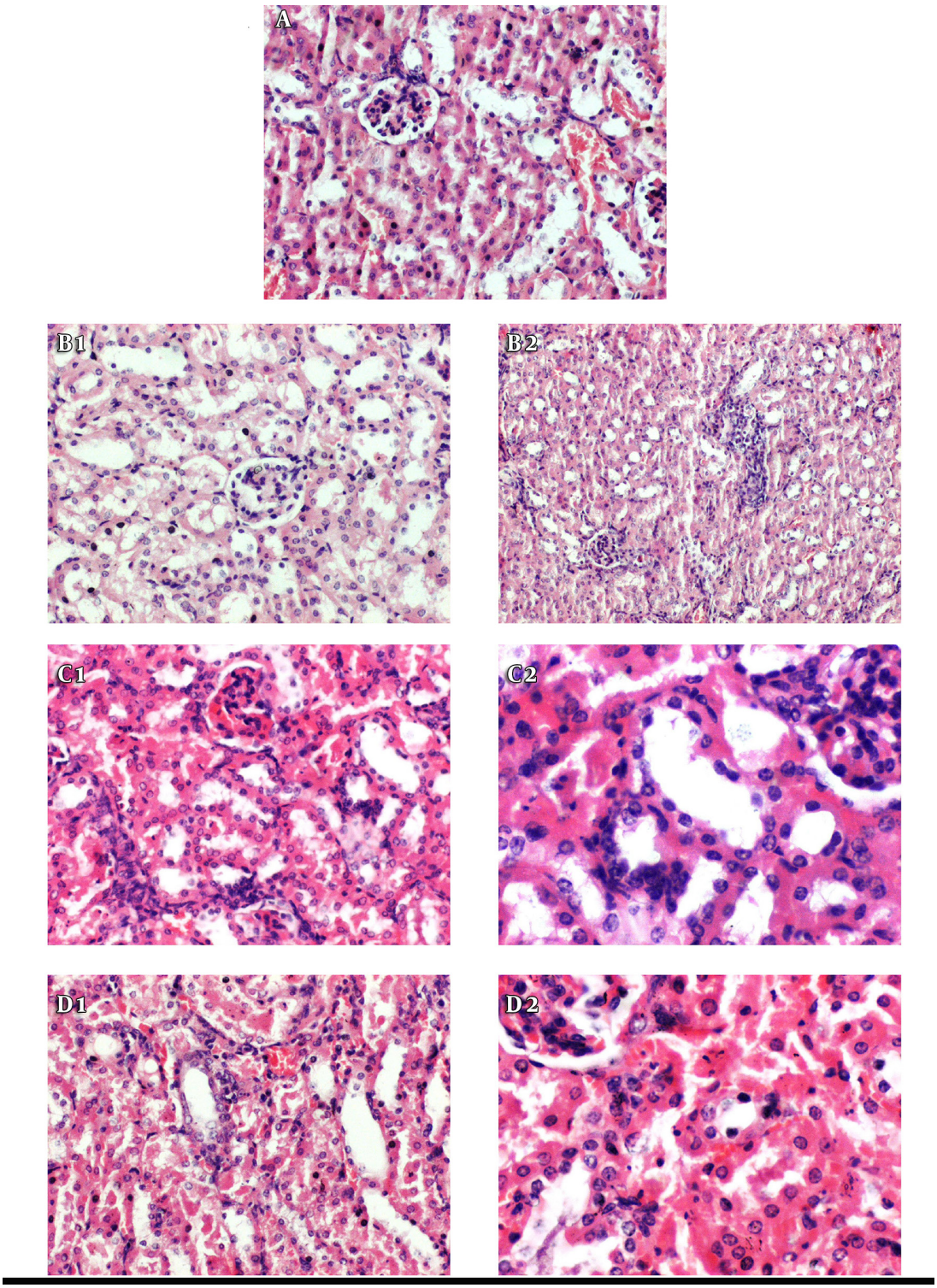
Figure 1. The Relation of Total Antioxidant Status and Total Oxidant Status Levels in Mice Renal Homogenate of the Study Groups

4.3. Pathological Evaluation

There were no pathological findings in any of the subjects in the control group. In all the intoxication groups, there were scarce-mild levels of lymphocytic infiltration and moderate-marked levels of hydropic degeneration. Starting from the low-dose α-AMA group (30% - 40%), loss of brush border became more eminent as the dose of intoxication increased; it was most intensely observed in the high-dose group (50% - 60%). Similarly, while areas of necrosis were < 15% in the low-dose α-AMA group, they were observed in 30% - 50% ratios in the high-dose group. Thyroidization signs were detected in four subjects in the high-dose α-AMA group (Figure 2).

Our results indicated that α-AMA poisoning in mice resulted in inflammatory changes and necrosis in renal structures. The development of necrosis and degeneration in renal tissues became more evident with increasing doses of intoxication.

Figure 2. The Pathological Findings With Hematoxylin and Eosin Staining Under the Light Microscope



(A) Normal renal tubular structure in the control group (H & E, X200). (B) Renal tubular cells with hydropic degeneration and tubules with loss of brush border (B1) (H & E, X200) and stromal lymphocyte infiltration (B2) (H & E, X100) in the low dose α-AMA group. (C) Renal tubular cells with more apparent hydropic degeneration, tubules with increasing loss of brush border and necrotic tubular epithelial cells (C1) (H & E, X200), necrotic tubular epithelial cells and loss of brush border (C2) (H & E, X400) in the moderate dose α-AMA group. (D) Renal tubular cells with most apparent hydropic degeneration, tubules with increasing loss of brush border and increment of necrotic tubular epithelial cells (D1) (H & E, X200), necrotic tubular epithelial cells and loss of brush border (D2) (H & E, X400) in the high dose α-AMA group.

5. Discussion

SOD, CAT and GSH-Px are the main enzymes in the cell to eliminate ROS (26). A significant increase of SOD activity in mice liver homogenates was reported after exposure to α-AMA (16). In an in-vitro experimental study, SOD activity was reported to be elevated in hepatocytes exposed to α-AMA (27). In another in-vivo experimental study involving mice hemolysate, α-AMA was responsible for a significant increase in SOD activity in all the study groups (24).

There are many experimental nephrotoxicity studies involving exposure to toxins and drugs. In an in-vivo experiment with rats using orellanine which is the toxin found in *Cortinarius* species mushrooms, oxidative stress was reported to play a role in toxin-related nephrotoxicity. Additionally, SOD activity and renal mRNA levels decreased in that study (28). In studies on nephrotoxicity involving cisplatin, a potent chemotherapeutic drug, the SOD activity was reported to be decreased together with other antioxidant enzyme levels (29, 30).

In our study, SOD activity in mice renal homogenate was significantly increased in moderate and high-dose α-AMA groups (P = 0.001 and P = 0.001, respectively). In our opinion, the increase in the SOD activity was due to the production of superoxide anion radicals, induced by ≥ 0.6 mg/kg α-AMA.

Studies in the literature report that GSH-Px is responsible for the elimination of H_2O_2 when its concentration is low. Then, CAT carries out this function in high H_2O_2 concentrations (31, 32). CAT is reported to have its highest activity in liver and kidney cells as well as in mammalian red blood cells. (16, 33). Following exposure to α-AMA, CAT activity in mice liver homogenate decreased, though this was not significant; in another study, CAT activity significantly reduced in hepatocyte culture medium (16, 27). However, in an in vivo experimental study using mice hemolysate, CAT activity increased with the increasing doses of α-AMA (24).

In two different experimental in-vivo studies that found relationship between drug-induced nephrotoxicity and oxidative damage, CAT activity decreased in renal structures (29, 34). In in vivo experimental rat studies involving cadmium and mercury, CAT levels also were reported to be decreased (35, 36).

In our study, we found that CAT activity in the renal cells exposed to α-AMA in vivo significantly reduced in the low- and high-dose groups, while there was no difference between the moderate-dose and the control groups (P = 0.006, P = 0.493, and P = 0.005, respectively). Our results suggest that CAT activity is suppressed in renal cells. Zheleva et al. stated that a possible explanation for in vivo and in vitro inhibitory effect of α-AMA on CAT in hepatocytes was the blockage of substrate presentation to the CAT Feheme pocket (16).

The GSH-Px activity decreased in both drug-induced and toxin-related experimental nephrotoxicity studies in the literature (29, 30, 35, 36).

Our study showed increased GSH-Px activity in mice renal homogenates in low- and moderate-dose α-AMA groups (P = 0.922 and P = 0.001, respectively). In the high-dose group, the GSH-Px activity significantly reduced (P = 0.001). If GSH-Px is responsible for the elimination of H_2O_2 in low-dose α-AMA exposures, the depletion of GSH-Px due to elimination of large amounts of H_2O_2 in case of > 0.6 mg/kg exposure may be the reason for these results.

One of the methods for the assessment of lipid peroxidation is measuring the levels of MDA products (19). Following the administration of α-AMA in sublethal doses, the MDA levels in mice liver homogenate decreased insignificantly at the 20th hour, but it increased significantly on the 6th day of intoxication (16). The levels of MDA products significantly increased in the hepatocyte medium exposed to α-AMA for 48 hours in vitro (27). On the other hand, the MDA products decreased insignificantly in mice hemolysate of all the intoxication groups following in vivo α-AMA exposure for 48 hours (24). The results of these studies, which have been carried out in different tissues both in vivo and in vitro, indicated that lipid peroxidation level after exposure to α-AMA was dependent on the effectiveness of the antioxidant defense system and the duration of exposure to the toxin.

MDA products significantly increased in in vivo rat studies investigating cisplatin, colistin and gentamicin-induced nephrotoxicity (29, 34, 37). Additionally, after exposure to mercury and cadmium in the in vivo rat model, the MDA product levels increased (35, 36).

In our study, the levels of MDA products significantly increased in mice renal homogenates following exposure to α-AMA in all the intoxication groups (P = 0.002, P = 0.001, and P = 0.002, respectively). This result indicated the development of peroxidative process in mice renal structures following exposure to α-AMA.

TOS and TAS measurements helped to eliminate cumbersome analysis of diverse oxidant and antioxidant molecules, which have additive effects. In a study involving patients with osteoarthritis, there was a statistically significant negative correlation between TOS and TAS (P < 0.001) (20, 21). There have also been different clinical studies on TAS and TOS measurements as an indicator of oxidative/antioxidative balance (38-40). In experimental studies investigating the relationship between oxidative stress following toxic exposure and nephrotoxicity, we observe that while the TOS level increased significantly in the presence of oxidative stress, the TAS level decreased significantly (41, 42). However, we could not find any studies in the literature investigating TAS and TOS activity in renal cells following in vivo α-AMA exposure.

In our study, the TOS levels significantly increased in mice renal homogenate following exposure to α-AMA (P = 0.002, P = 0.001, and P = 0.001, respectively). As the dose of α-AMA increased, the TOS levels showed significant increases accordingly (P = 0.034, P = 0.001, and P = 0.041, respectively). The TAS levels showed a significant increase in the low-dose α-AMA group compared to the

control group; however, it significantly reduced in the moderate and high-dose groups (P = 0.031, P = 0.001, and P = 0.001, respectively). These results indicated that oxidative/anti-oxidative balance shifts towards oxidative status in groups exposed to ≥ 0.6 mg/kg α-AMA and oxidative stress develops in mice renal structures (Figure 1). Possible reasons for this finding may be acting of the toxin as free radical, or its metabolism to free radical, excess ROS production due to toxin metabolism, or the antioxidant property of the toxin. We believe further studies are necessary to understand the mechanism of the effect of α-AMA on renal cells.

The strong point of our study was the intoxication model in which there was no death after α-AMA IP was injected. We got the results from all the animals, so we did not have any data loss. However, our study did not evaluate the changes of those biomarkers over time after exposure, which was the weak point.

The pathology results of our study showed the presence of renal damage in in vivo mice intoxication model, arranged with α-AMA injection, while the results of oxidative stress biomarkers indicated the development of oxidative stress in mice renal structures. We believe that our findings provide a basis for research on the pathophysiology of renal damage caused by α-AMA intoxication and treatment modalities aiming to enhance antioxidative system and prevent damage.

Pharmacokinetics and pharmacodynamics of ingested α-AMA orally may differ from injected α-AMA IP. An animal model may not represent the same antioxidant response seen in human beings.

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Authors' Contributions

Study concept and design: Mehmet Ergin, Zerrin Defne Dundar, Ibrahim Kilinc. Acquisition of data: Mehmet Ergin, Zerrin Defne Dundar, Ibrahim Kilinc, Tamer Colak, Pembe Oltulu. Analysis and interpretation of data: Mehmet Ergin, Zerrin Defne Dundar, Ibrahim Kilinc, Pembe Oltulu, Abdullah Sadik Girisgin. Drafting of manuscript: Mehmet Ergin, Zerrin Defne Dundar, Tamer Colak, Abdullah Sadik Girisgin. Critical revision of manuscript for important intellectual content: Mehmet Ergin, Zerrin Defne Dundar, Abdullah Sadik Girisgin. Statistical analysis: Mehmet Ergin, Zerrin Defne Dundar. Administrative, technical and material suport: Mehmet Ergin, Zerrin Defne Dundar, Tamer Colak. Study supervision: Ibrahim Kilinc, Abdullah Sadik Girisgin.

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