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Post-Mortem Changes in Acetylcholinesterase, Na+/K+-ATPase, Phosphofructokinase, Glutathione Peroxidase, Catalase, and Superoxide Dismutase Activities in Rats Exposed to Toluene

Tolüene Maruz Kalan Sıçanlarda Asetilkolinesteraz, Na+/K+-ATPaz, Fosfofruktokinaz, Glutatyon Peroksidaz, Katalaz ve Süperoksit Dismutaz Aktivitelerinde Post-Mortem Değişiklikler

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ABSTRACT

Objective: Exposure to high doses of toluene, which is widely used in industry, not only causes various symptoms, but can also result in death. The aim of this study is to investigate post-mortem changes in Acetylcholinesterase, Na+/K+-ATPase, Phosphofructokinase and antioxidant enzymes (Glutathione Peroxidase, Catalase, and Superoxide Dismutase) in rats exposed to toluene and to reveal possible post-mortem clues.

Methods: Rats were injected with a lethal dose of toluene. Blood, brain and liver tissue were excised at 0, 6, 12, 24 and 48 hours after death. Enzyme activities were assayed from the supernatants prepared.

Results: The enzyme activities of toluene groups have been decreased in comparison with the control group for both AChE and PFK enzymes, on the other hand, there was an increase in the Na+/K+-ATPase activity in the brain tissues. In antioxidant enzymes, increases in activity of all 3 enzymes were generally observed when compared to the control group. Plasma GSH-Px activity was observed to decrease between 0-12 hours post-mortem and to increase at 24 and 48 hours post-mortem. GSH-Px activities in the liver showed significant increases at 0, 12 and 48 hours. CAT activities in liver distinctly increased. Significant differences in SOD levels were not detected when compared with the control. Oxidative damage occurring in cells under the effect of toluene may have disrupted the membrane structure, the three-dimensional structure of proteins and lipids, and the cell repair mechanisms.

Conclusion: Consequently, it was determined that oxidative damage due to toluene may cause changes in some enzyme activities in the post-mortem period, which may prove oxidative damage.

Keywords: Post-mortem, biochemistry, antioxidants, enzymes, toluene

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ÖZ

Amaç: Endüstride yaygın olarak kullanılan toluene yüksek dozda maruz kalınması çeşitli semptomlara yol açabileceği gibi ölümle de sonuçlanabilmektedir. Bu çalışmanın amacı, toluene maruz kalan sıçanlarda Asetilkolinesteraz, Na+/K+-ATPaz, Fosfofruktokinaz ve antioksidan enzim aktivitelerindeki (Glutatyon Peroksidaz, Katalaz ve Süperoksit Dismutaz) post-mortem dönemdeki değişiklikleri araştırmak ve ölüm sonrasına ilişkin olası ipuçlarını ortaya koymaktır.

Yöntem: Çalışmada letal dozda toluen enjekte edilen sıçanlar ölümden 0, 6, 12, 24 ve 48 saat sonra disekte edilerek kan, beyin ve karaciğer dokusu toplandı. Hazırlanan süpernatanlardan enzim aktiviteleri analiz edildi.

Bulgular: Beyin dokularında, toluen uygulanan deney gruplarının enzim aktiviteleri hem AChE hem de PFK enzimleri için kontrol grubuna göre azalırken Na+/K+-ATPaz aktivitesinde artış bulundu. Antioksidan enzimlerde kontrol grubu ile karşılaştırıldığında genel olarak üç enzimin de aktivitesinde artış gözlendi. Plazma GSH-Px aktivitesinin ölümden 0-12 saat sonra azaldığı, 24 ve 48 saat sonra ise arttığı gözlendi. Karaciğerde GSH-Px aktiviteleri 0, 12 ve 48. saatlerde önemli artışlar gösterdi. Karaciğerde CAT aktivitesi belirgin şekilde arttı. SOD seviyelerinde önemli farklılıklar tespit edilmedi. Toluen etkisi nedeniyle hücrelerde meydana gelen oksidatif hasarın, zar yapısını, protein ve lipidlerin üç boyutlu yapısını ve hücre onarım mekanizmalarını bozduğu düşünülmektedir.

Sonuç: Sonuç olarak, bu çalışmada tolüene bağlı oksidatif hasarın post-mortem süreçte bazı enzim miktarlarında değişikliğe yol açabileceği bunun da oksidatif hasarı kanıtlayabileceği belirlendi.

Anahtar Kelimeler: Post-mortem, biyokimya, antioksidan, enzim, toluen

INTRODUCTION

In recent years, post-mortem biochemistry has become an important field of interest, especially in forensic science, for determining causes of death and post-mortem interval (PMI) (1). Post-mortem biochemical studies contribute to the understanding of biochemical changes occurring in organisms. The level of biochemical substances in bodily fluids changes according to PMI and cause of death (2). In this field of study, post-mortem changes in hormones, electrolytes, enzymes and various molecules of such as glucose, creatinine and uric acid are generally investigated (3). For example, it was found that levels of the liver enzyme gamma glutamyl transferase (GGT) increased in the death process and that there was a mild increase in levels of serum amylase (AMY) and GGT in hypothermia (3). In another study that investigated changes in electrolyte in the post-mortem period, it was revealed that cold environmental conditions affected potassium levels in the vitreous humor, and that in subjects exposed to a cold chamber, there was a negative correlation between sodium and chloride levels of vitreous humor with time since death (4). Moreover, sodium-potassium ATPase, acetylcholinesterase (AChE) and glutathione S-transferase activities were examined to determine PMI (5). In this study, toluene was used to examine its post-mortem effects on different enzymes; AChE, sodium-potassium ATPase (Na+/K+-ATPase), phosphofructokinase (PFK), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD).

Toluene ($C_6H_5CH_3$) is a volatile aromatic compound with a characteristic smell (6). It is one of the organic solvents widely used in industry, and can be found in paints, varnishes, adhesives, solvent-based cleaning fluids, and numerous household products and cosmetics (7). The amount of toluene in paint thinner sold in Turkey is approximately 50-70% (8). Moreover, since it is easily obtainable, it is one of the most widely abused solvents. According to researchers, 90% of volatile substance abusers use toluene, although substances such as butane, n-hexane, trichloroethane, benzene, methyl butyl ketone and trichloroethylene are also substances abused through inhalation (9).

The first step in toluene metabolism is hydroxylation and oxidation reactions catalyzed by cytochrome p450, as a result of which, reactive oxygen species (ROS) are formed and oxidative stress is observed (10). Organic solvents such as toluene have toxic effects on cells and organs due to ROS (11). Furthermore, organisms possess an antioxidant defense system containing the antioxidant enzymes CAT, GSH-Px and SOD for reducing the harmful effects of ROS (12). Researchers have revealed the changing levels of these enzymes due to exposure to volatile substances like toluene and paint thinner (13-15).

In addition to the effects of toluene on antioxidant enzymes and on various body systems, according to studies conducted, toluene causes toxic effects on the brain and central nervous system (CNS) (16). As for the Na+/K+-ATPase and according to some studies, toluene has not been found to cause toxic effects on the blood, and red blood cell membranes exposed to toluene have been found to exhibit different behaviors against lysis (16). Regarding the PFK enzyme and according to most studies showed that the cause of decreases in enzyme activity in the test group compared to the control group thought to be the toxic effects of toluene on the brain (17).

The aim of this study is to evaluate post-mortem changes in levels of enzymes (CAT, SOD, GSH-Px, AChE, Na+/K+-ATPase, PFK) for toluene-induced deaths in rats.

MATERIALS and METHODS

Chemicals

Toluene (97%), ethylenediaminetetraacetic acid (EDTA), potassium dihydrogen phosphate (KH_2P0_4), dipotassium hydrogen phosphate (K_2HPO_4), and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich Inc. (USA). GSH-Px, CAT and SOD assay kits were purchased from Cayman Chemical Company (Michigan, ABD), item numbers: 703102, 707002 and 706002, respectively. Cat No. ab138871 (AChE) Abcam, mbs2540486 (Na+/K+-ATPase) Mybiosource, mbs8243182 (PFK) Mybiosource.

Animals

This study was designed with male rats in order to prevent the interaction of hormonal fluctuations of female rats on the experimental parameters. Male Wistar rats weighing 350 g were used in the study, and the animals were kept in polycarbonate cages under the conditions of the Experimental Animal Breeding and Research Centre. Water and food were provided ad libitum at 22-24 °C under a 12-hour light-dark cycle. For each post-mortem period (0, 6, 12, 24 and 48 hours), rats were assigned to the control (n=10) and toluene groups (n=20). Rats were starved for 24 hours prior to experiments (18).

Exposure to Toluene

The intraperitoneal (IP) LD_{50} dose of toluene for rats is 1.332 g/kg (19). Following 24-hour starvation, the lethal dose of toluene was administered to the toluene group by IP injection, and as a result, mortality due to toluene poisoning occurred. In the control group, physiological saline solution was injected instead of toluene, and animals in the control group were euthanized by cervical dislocation. Animals in the toluene group died spontaneously 5-6 hours after administration of the lethal dose of toluene. Animals in the control group were taken for experiment simultaneously with animals in the toluene group.

Collection of Tissue and Plasma Samples

Brain and liver tissues, and plasma samples were prepared by following the sample preparation instructions for the assay kits used for measuring enzyme activity. Plasma samples and tissues were collected for each rat at 0, 6, 12, 24 and 48 hours post-mortem. To prevent clotting, plasma samples were collected in tubes with EDTA and immediately centrifuged at 1.000 g for 10 minutes in a Hermle Z 326 K centrifuge. Plasma samples were kept at -80 °C until assay. To remove red blood cells and clots, liver and brain tissues were washed with cold phosphate-buffered saline (pH=7.4) and then homogenized in 9 mL cold buffer per gram of tissue in a frozen state in a Schuett homogen-plus homogenizer at 3.000 rpm. All homogenates were centrifuged, at 10,000 g for 15 minutes to assay GSH-Px and CAT activity, at 1.500 g for 5 minutes to assay SOD activity, at 2.500 rpm/min for 5-10 min to assay AChE activity, and at 8.000 g for 10min to assay Na+/K+-ATPase and PFK activities. Following the centrifugation processes, supernatant was separated from the pellet and kept at -80 °C until analysis.

Measurement of Enzyme Activities

AChE, Na+/K+-ATPase, PFK, GSH-Px, SOD, and CAT activities were measured using an assay kit by colorimetric measurement in blood, brain, and liver tissue samples. All analyses were made in accordance with the kit instructions. AChE Assay is based on an improved Ellman method, in which thiocholine produced by the action of AChE forms a yellow color with 5,5'-dithiobis (2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm. ATPases assay is based on the formation of colored complexes between an inorganic phosphate and a dye molecule under acidic conditions. Such assays are beset with problems of reagent precipitation and high backgrounds caused by impure substrates (i.e. contaminated with inorganic phosphate) and/or by non-enzymatic (acid) hydrolysis of the substrate. The absorbance values were recorded at 636 nm (novusbio 601-0120), PFK assay based on, PFK converts fructose-6-phosphate and ATP to fructose-diphosphate and ADP. The ADP in the presence of substrate and enzyme mix is converted to AMP and NADH, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. The absorbance values were recorded at 340 nm (Abnova KA3761). The SOD assay is based on detection of superoxide radicals produced by xanthine oxidase and hypoxanthine, and tetrazolium salt reacts with superoxide anions. At the end of the reaction period, absorbance values were recorded at 460 nm (BioTek ELx800) (20). GSH-Px activity was measured according to the Lawrence and Burk method, and changes in absorbance were recorded at 340 nm during oxidation of NADPH to $NADP^+$ (21). CAT activity was assayed by recording changes in absorbance at 540 nm of released formaldehyde based on reaction with methanol in the presence of H_2O_2 (22,23).

Statistical Analysis

Statistical analysis of the data was made using the SPSS 24.0 software program. To compare the effects of toluene between experimental and control groups, independent samples t-test was used. To examine changes in post-mortem period one-way analysis of variance (ANOVA) was performed. Differences in post-mortem period (0, 6, 12, 24 and 48 hours) between groups were evaluated with multiple comparison tests. Post-hoc tests were used for multiple comparisons and results were analyzed according to Tukey HSD test. Data were presented in histograms as mean and standard deviation values. Data were accepted as significant at $p<0.05$.

RESULTS

GSH-Px Results

Changes in liver tissue and plasma GSH-Px activity are shown with statistical data in Figure 1. In the liver, GSH-Px activities of toluene groups were found to be higher than those of control groups ($p<0.05$). While there was a decrease in activity in the control group after 12 hours, a significant change in liver GSH-Px activity based on PMI was not observed in the toluene group ($p>0.05$) (Figure 1a).

In plasma samples of the group given toluene, while GSH-Px activity significantly decreased up to 12 hours post-mortem, an increase in activities was seen after 12 hours ($p<0.05$) (Figure 1b). Moreover, statistically significant increases were observed in toluene groups when compared to control groups ($p<0.05$). It is striking that for both liver samples and plasma samples, GSH-Px activities in the control group reached the lowest level at the 12th hour.

SOD Results

Statistical data and SOD activity values are given in Figure 2. The obtained data showed increases and decreases between groups, but a statistically significant difference was not recorded for changes in activity of groups related to PMI (Figure 2a and 2b). When compared with control groups, an increase in SOD activity was seen in toluene groups.

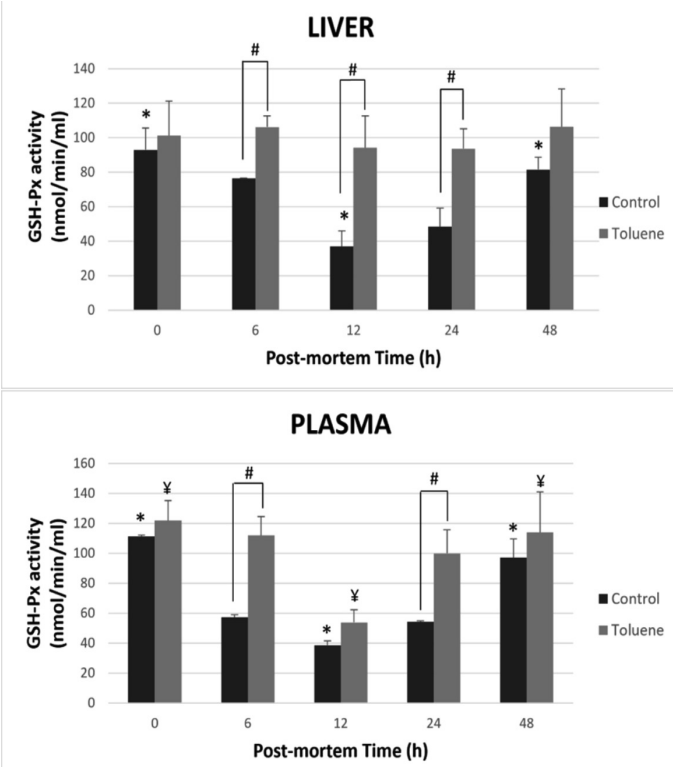

Figure 1. GSH-Px activity values in liver tissue (a) and plasma (b), in relation to post-mortem time. (#) indicate that statistically significant difference between control and toluen group. In addition, the data indicated by (*) and (¥) are statistically significant within the groups according to the post-mortem time ($p<0.05$)

CAT Results

CAT activities in liver tissue and plasma are presented in Figure 3. It can be seen that in the group administered toluene, enzyme activities in the liver increased compared to the control group. These increases were statistically significant over time ($p<0.05$) (Figure 3a). In addition, results show that increasing CAT activity after 6 h.

As for activity values in plasma between groups, an increase over time, albeit small, was seen in the control group. In the group administered toluene, however, despite a decrease at the 6th hour, statistically insignificant increases were observed at the 12th, 24th and 48th hours ($p>0.05$) (Figure 3b).

AChE Results

Changes in brain tissue and blood AChE activity are shown with statistical data in Figure 4. In the brain, AChE activities of toluene groups were found to be lower than the control groups. These decreases were statistically significant over time ($p<0.05$) (Figure 4a). In the blood, AChE activities of toluene groups were found to be lower than the control groups and these increases were statistically insignificant over time ($p>0.05$) (Figure 4b).

Na+/K+-ATPase Results

Changes in brain tissue and blood Na+/K+-ATPase activity are shown with statistical data in Figure 5. In the brain, the activity

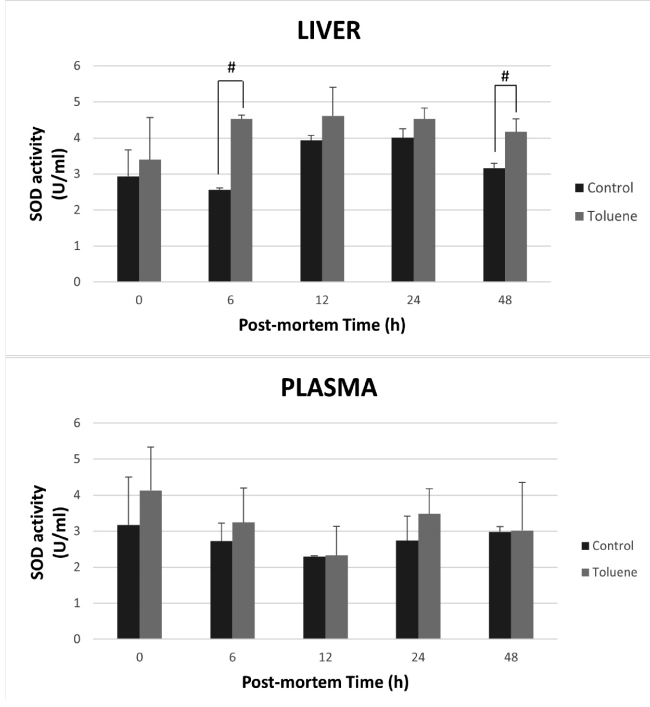

Figure 2. SOD activity values in liver tissue (a) and plasma (b), in relation to post-mortem time. (#) indicate that statistically significant difference between control and toluen group ($p<0.05$)

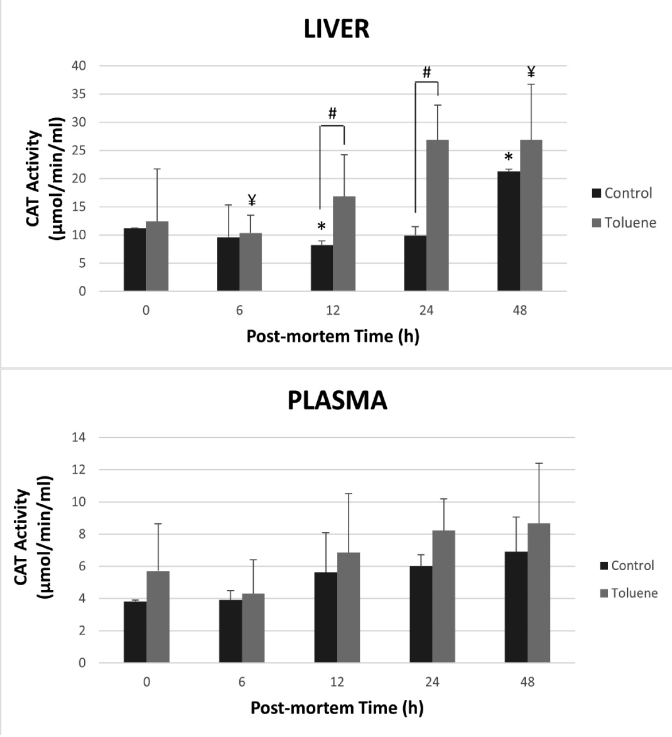

Figure 3. CAT activity values in liver tissue (a) and plasma (b), in relation to post-mortem time. (#) indicate that statistically significant difference between control and toluen group. In addition, the data indicated by (*) and (¥) are statistically significant within the groups according to the post-mortem time ($p<0.05$)

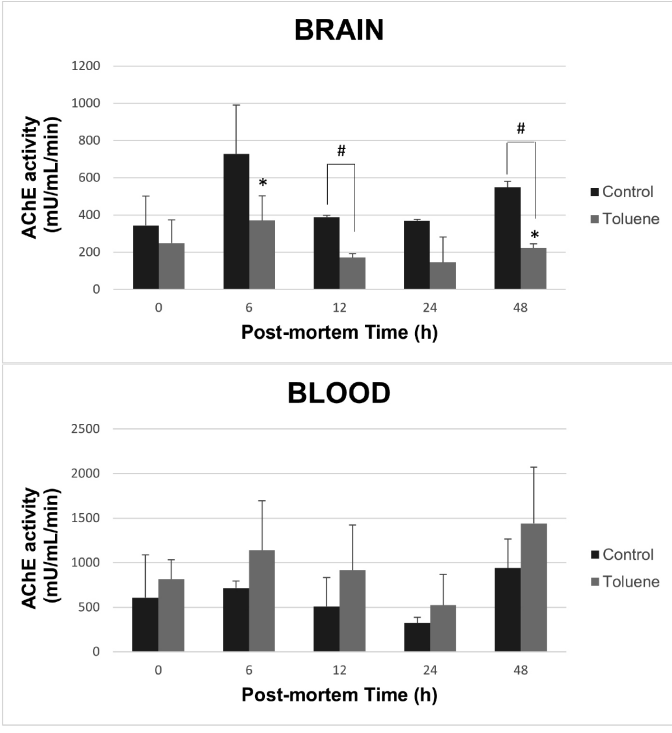

Figure 4. AChE activity values in liver tissue (a) and plasma (b), in relation to post-mortem time. (#) indicate that statistically significant difference between control and toluen group. In addition, the data indicated by (*) are statistically significant within the group according to the post-mortem time ($p<0.05$)

of the toluene group was statistically significantly higher than the control group ($p<0.05$), only at 12 and 24 hours weren't significant ($p>0.05$). A statistically significant decrease was observed in the control group from 12 hours to 48 hours ($p<0.05$) (Figure 5a).

As for activity values in blood between groups, Na+/K+-ATPase activities in blood are given in (Figure 5b). In the blood at 12 hours only, the activity of the toluene group was statistically significantly higher than the control group ($p<0.05$) (Figure 5b).

PFK Results

PFK activities in liver tissue and plasma are presented in Figure 6. In the brain, the activity of the toluene group was statistically significantly lower than the control group ($p<0.05$), only at 6 hours the activity wasn't statistically significant ($p>0.05$) (Figure 6a). Also, statistically significant decrease was seen in control group after 6 h.

The effects of toluene on the PFK activities of plasma tissues are given in (Figure 6b). In the plasma at 0 hours, the activity of the toluene group was statistically significantly higher than the control group ($p<0.05$). When post-mortem time-related activity changes are investigated, a statistically significant decrease was observed in toluene group and increase in control groups from 0 hours until 48 hours ($p<0.05$).

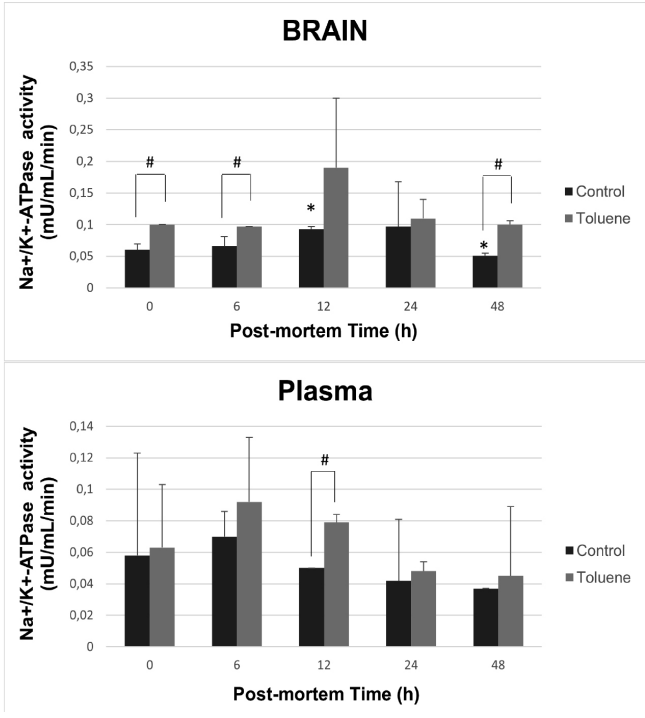

Figure 5. Na+/K+-ATPase activity values in liver tissue (a) and plasma (b), in relation to post-mortem time. (#) indicate that statistically significant difference between control and toluen group. In addition, the data indicated by (*) are statistically significant within the groups according to the post-mortem time ($p<0.05$)

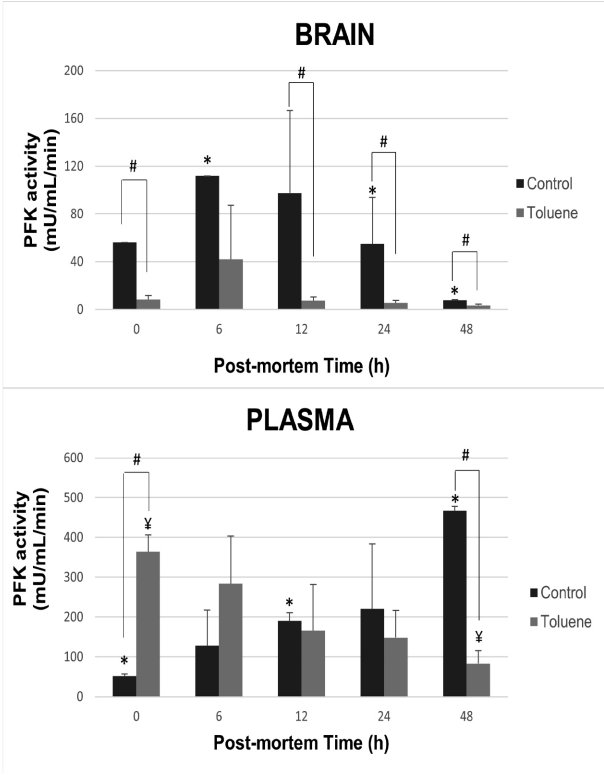

Figure 6. PFK activity values in liver tissue (a) and plasma (b), in relation to post-mortem time. (#) indicate that statistically significant difference between control and toluen group. In addition, the data indicated by (*) and (¥) are statistically significant within the groups according to the post-mortem time ($p<0.05$)

DISCUSSION

Toluene is a volatile organic compound that is widely used in industry and household products, as well as being one of the solvents that are frequently abused. Not only does toluene cause harmful effects on health, but also, exposure to toluene in high doses can result in death. In the studies carried out by Ameno et al. (24) and Akcan et al. (25), deaths due to acute toluene exposure or to paint thinner were reported. Toluene causes oxidative stress in cells. Antioxidant enzymes in the metabolism are special enzymes that respond to oxidative stress (12).

A number of studies examining the effects of toluene on antioxidant enzymes are based on chronic exposure (13,14,26). In our study, however, by investigating the post-mortem effect of toluene, data were examined that would offer an insight into determining the reason for death. The results of the study reveal that exposure to a lethal dose of toluene resulted in increases in antioxidant enzyme activities in rat liver and plasma samples in post-mortem periods. These increases in enzyme activities may be due to ROS production by the cytochrome p450 family of enzymes, which is the first step of toluene metabolism, especially in the liver (11). It has been reported that ROS formation and lipid peroxidation in various tissues increase due to toluene and that for this reason, toluene exhibits toxic effects on cells (27,28). If there is a lack of balance between free radicals and antioxidants in the defense against oxidative stress, oxidative damage to cells and tissues occurs in organisms (26). Oxidative damage especially affects levels of molecules such as antioxidant enzymes and vitamins (29).

On the other hand, sudden deaths due to exposure to toluene may occur because of multiple organ damage, such as cardiovascular effects like myocardial ischemia or infarction, cardiomyopathy, myocarditis; neurotoxicity or irreversible structural changes in the brain; or hypoxemia or asphyxia in the lungs (8). Furthermore, toluene inhalation can cause serious damage to the liver depending on the period of exposure, such as focal necrosis, granuloma, necrosis and portal inflammation (30).

After exposure to toluene, increases in antioxidant enzyme activities were observed. We also asked the question, "How do these enzyme activities vary according to post-mortem time?" and examined the results. The most interesting finding was that although GSH-Px activity in both liver and plasma firstly decreased between 0-12 hours, it increased after 12 hours. Similarly, after 6-12 hours post-mortem, CAT activity in the liver significantly increased.

With complete and irreversible cessation of circulation and respiration, somatic death occurs within several minutes after death. Later, molecular death generally occurs within 1-2 hours with the death of tissue and cells and the termination of vital functions (31). After death, due to lack of oxygen in circulation and cessation of anabolic reactions of metabolites, the occurrence of certain biochemical changes in organisms is expected (32). Accordingly, GSH-Px activity may show a reduction during the first time period after death.

On the other hand, increases in enzyme activity may occur due to the process of autolysis, especially between 12 and 48 hours post-mortem. A possible explanation for this may be that as a result of autolysis, as post-mortem time increases, enzymes may be released from cells due to destruction of cell and tissue structures. Moreover, when cells are exposed to toxic substances such as toluene, they respond by increasing their antioxidant levels. Due to antioxidant consumption in body reserves, total antioxidant capacity may decrease with enzymes. For this reason, an imbalance in activities may occur.

In a study conducted by Sharma et al. (33), various enzyme activities were investigated in terms of PMI (<6 hours, 6-12 hours and 12-24 hours), such as levels of AMY, GGT, lactate dehydrogenase and creatine kinase (CK). As a result of the study, a positive correlation of all four enzymes with PMI was observed. Moreover, it was revealed that the increase in CK activity was statistically significant. As a result, it was

hypothesized that in the post-mortem autolysis process, cardiac enzymes were released into pericardial fluid and that levels of cardiac enzymes may increase after death (33).

Considering these results, the fact that antioxidant enzyme activities in the toluene group increased in comparison with the control group in our study gives rise to the thought that toluene increases oxidative stress. In addition, it seems that antioxidant enzyme activities, especially GSH-Px, tend to decrease in the early post-mortem period and to increase after 12 hours.

Besides antioxidant enzymes, we investigated how Na+/K+-ATPase, PFK, AChE activities were affected by toluene in both brain tissues and blood (plasma) of heart samples in the post-mortem periods. This effect was observed as a significant increase in brain Na+/K+-ATPase activity at post-mortem 0, 6 and 48 hours, and in blood, it was at post-mortem 12hours. In contrast, AChE and PFK activities in brain decreased at post-mortem time.

According to studies conducted, toluene causes toxic effects on the brain and CNS (16). In the most of inhalation studies, neurological effects such as brain weight reduction, changes in concentrations of neurotransmitters and neurobehavioral performance deficits have been reported in chronically exposed animals (34,35). Similarly, in studies on rats, the effects of toluene inhalation on brain components, morphological and biochemical parameters were determined. At the end of the study, rats exposed to dose levels approaching 2000 ppm for 7 days caused ataxia, prostration and shivering (36).

In a study carried out by Korpela and Tahti (37), the mechanism of the anesthetic effect of toluene on the CNS, both in vitro and in vivo, has been investigated for synaptic membranes. An inhibitory effect on AChE enzyme activity was shown in rats exposed to doses of toluene for 2000 ppm. At the end of the study, toluene was determined to cause similar enzyme inhibitions in nerve cell membranes (37).

Similar results have been shown in the study done by Naalsund (38). A regional loss of cells accompanied by abnormal electrical activity was observed in the hippocampus region when rats were exposed to 500 ppm of toluene for 8-16 hours/day for up to 5 weeks. The result is that toluene causes irreversible effects in this region of the brain (38).

In an inhalation study and in a study related to the clinical and experimental neurotoxicity of toluene in rats, toluene was found to be present in all regions of the brain of tumors inhaled by rats, with the highest concentration of toluene in the brainstem region, and with neurological sequelae in toluene users (39). In another similar study, toluene absorption showed a clear correlation with fat content in each region of the brain. Enzyme activities and receptor binding were found to be the most affected by brainstem in rats exposed subchronic and chronic to toluene. The lack of neurological behavioral performance has been attributed to the toxic effects of toluene in the brain (40).

As for the Na+/K+-ATPase and according to some studies, toluene has not been found to cause toxic effects on blood, and red blood cell membranes exposed to toluene have been found to exhibit different behaviors against lysis (16).

According to the study made by Korpela, it has been found that red blood cell membranes of the test group were more potent and less susceptible to lysis when exposed to 2,000 ppm of toluene when compared to the control group, as a result of the toluene inhalation exposure study conducted in rats (41).

In another similar study, Korpela and Tahti (42) have shown that the characteristics of red blood cells membranes can be reversed according to the results obtained from the study that they were doing about the effects of toluene inhalation on rats. Their study has shown that the reason was that because the effect of toluene has been removed from the system and it has been found that the membrane power returns to normal (42).

In another study, the mechanism of anesthetic effect on toluene's CNS was investigated using rat red blood cells and synaptosomal membranes. In rats exposed to doses of toluene of 2000 ppm for a short time, an inhibitory effect was detected in total ATPase and Mg2 + -ATPase enzyme activities, contrary to the results of our study. At the end of the study, it was determined that toluene caused similar enzyme inhibitions in red blood cell membranes (37).

Regarding the PFK enzyme and according to most studies that showed that the cause of decreases in enzyme activity in the test group compared to the control group thought to be the toxic effects of toluene on the brain. On the other hand, most studies show that toluene is not a significant effect on blood. In the study conducted by Burns (17), erythrocytes, hemoglobin, hematocrit, leukocytes, RBC mean volume (MCV) and blood volume were compared in the control group and the experimental group in female rats exposed to 600 mg/kg/day of toluene by gavage for 14 days. There was no change in hemoglobin mean volume (MCH) (17).

In terms of clarifying the reasons for the decrease in enzyme activity of the brain and blood tissue, some studies have shown that extensive biochemical changes in whole body tissues after death (32). Within 1-2 hours, the death of tissues and cells occurs (31). According to one study, blood samples from animal corpses (rat and pig) were examined for 96 hours to investigate changes in blood pH and concentrations of six metabolites (lactic acid, hypoxanthine, uric acid, ammonia, NADH and formic acid). pH and concentrations of the metabolites had changed with post-mortem times, but the rate and rate of change were different (32). Oxygen deficiency, enzymatic reactions, cellular deterioration, blood pH and concentrations of six metabolites (lactic acid, hypoxanthine, uric acid, ammonia, NADH and

formic acid) may be the reasons for the changes in the AChE, Na + / K + ATPase and PFK enzymes activity in the brain and blood tissues at post-mortem times.

The increase in the activity of some serum enzymes can be considered due to the effect of hemolysis. For this reason, we believe that the PFK activity in the period after death 0-24 and 0-48 hours for plasma tissue may be due to the hemolysis. Similarly, in the study by Garg and Vidya Garg (43), serial quantitative changes in the levels of various serum enzymes were examined according to postmortem time intervals, and as a result, increases in glucose-6-phosphate dehydrogenase (G6PD) and acid phosphatase (APs) activities were observed.

CONCLUSION

Finally, it should not be forgotten that enzyme responses may vary depending on the amounts, nature and activities of enzymes in different tissues (44). Providing those experimental studies are improved with further studies with longer PMIs (such as 60 and 72 hours), the monitoring of GSH-Px levels in plasma, in particular, may assist post-mortem biochemical studies.

Ethics

Ethics Committee Approval: This study was conducted with the ethics committee decision (2016-11/03) of Bursa Uludağ University's Experimental Animal Ethics Committee.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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