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Investigation of The Effect of Artemisinin Administration on Total Oxidant/Antioxidant and Oxidative Stress-Index in The Liver and Kidney Tissue of Pentylenetetrazole-Induced Mice

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ABSTRACT This study was designed to investigate the changes in total oxidant (TOS)/antioxidant (TAS) and oxidative stress index (OSI) levels in liver and kidney tissues of mice pre-treatment of artemisinin against oxidative stress that may occur in mice administered pentylenetetrazole (PTZ). Swiss albino mice (Male) (n=42) were used in the study. The mice were divided into six groups and each group had seven animals (n=7): (1) Control (C) /saline Group, (2) PTZ (35 mg/kg) Group, (3) Valproate (VPA) (100 mg/kg) + PTZ Group, (4) Artemisinin (ART) (30 mg/kg) + PTZ Group, (5) ART (60 mg/kg) + PTZ Group, (6) ART (120 mg/kg) + PTZ Group. Mice received injections intraperitoneally (ip). After the treatments, the animals were observed for seizures for 30 minutes. On the last day (day 26) of the experiment, the PTZ loading dose (75 mg/kg) was administered to the mice and then the animals were sacrificed. TAS, TOS and OSI levels were measured in liver and kidney tissue. PTZ increased TOS and decreased TAS in liver and kidney tissue. ART significantly increased TAS and decreased TOS in liver tissue at increasing doses ($p<0.05$). ART was not very effective in kidney tissue. However, TAS levels in kidney tissue were significantly higher when VPA was compared with other groups ($p<0.05$). In this study, it can be assumed that PTZ-induced oxidative stress may be due to the activation of glutamate receptors in peripheral tissues. ART may have a protective effect against liver damage due to PTZ-induced oxidative stress and hypoxia. This effect may be due to the antioxidant capacity of ART.

Keywords: *Artemisinin, Kidney, Liver, Oxidative stress, Pentylenetetrazol.*

ÖZ

Artemisinin Uygulamasının, Pentilentetrazol ile İndüklenen Farelerin Karaciğer ve Böbrek Dokusunda Total Oksidan/Antioksidan ve Oksidatif Stres İndeksi Üzerine Etkisinin Araştırılması

Bu çalışma, pentilentetrazol (PTZ) uygulanan farelerde oluşabilecek oksidatif strese karşı artemisinin ön tedavisinin farelerin karaciğer ve böbrek dokularında toplam oksidan (TOS)/antioksidan (TAS) ve oksidatif stres indeksi (OSI) düzeylerindeki değişiklikleri araştırmak için tasarlanmıştır. Çalışmada İsviçre albino fareleri (Erkek) (n=42) kullanıldı. Fareler altı gruba ayrıldı ve her grupta yedi hayvan (n=7) vardı: (1) Kontrol (C) /salin Grubu, (2) PTZ (35 mg/kg.) Grubu, (3) Valproat (VPA) (100 mg/kg) + PTZ Grubu, (4) Artemisinin (ART) (30 mg/kg) + PTZ Grubu, (5) ART (60 mg/kg) + PTZ Grubu, (6) ART (120 mg/kg) + PTZ Grubu. Fareler, intraperitoneal (ip) olarak enjeksiyonlar uygulandı. Uygulamalardan sonra hayvanlar 30 dakika boyunca nöbetler için gözlendi. Deneyin son gününde (26. gün) farelere PTZ yükleme dozu (75 mg/kg) uygulandı ve ardından hayvanlar sakrifiye edildi. Karaciğer ve böbrek dokusunda TAS, TOS ve OSI düzeyleri ölçüldü. PTZ, karaciğer ve böbrek dokusunda TOS'u artırdı ve TAS'ı azalttı. ART, artan dozlarda karaciğer dokusunda TAS'ı önemli ölçüde artırdı ve TOS'u azalttı ($p<0.05$). ART böbrek dokusunda çok etkili değildi. Ancak böbrek dokusundaki TAS düzeyleri VPA diğer gruplarla karşılaştırıldığında anlamlı olarak yüksekti ($p<0.05$). Bu çalışmada, PTZ kaynaklı oksidatif stresin, periferik dokularda glutamat reseptörlerinin aktivasyonundan kaynaklanabileceği varsayılabilir. ART, PTZ'nin neden olduğu oksidatif stres ve hipoksiye bağlı karaciğer hasarına karşı koruyucu bir etkiye sahip olabilir. Bu etki, ART'nin antioksidan kapasitesine bağlı olabilir.

Anahtar Kelimeler: *Artemisinin, Böbrek, Karaciğer, Oksidatif stres, Pentilentetrazol.*

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INTRODUCTION

It is known that seizures due to epilepsy cause oxidative stress (OS) not only in the nervous system but also in other systems of the organism (Hamed 2017). OS is caused by the imbalance in the oxidant/antioxidant defense system of the organism as a result of the overproduction of free radicals (Demirci-Çekiç et al. 2022). This imbalance contributes to the formation of various diseases in the cardiovascular, digestive and nervous systems (Bhatti et al. 2022). Epilepsy is one of the neurodegenerative diseases that affect people worldwide. This disease can increase the formation of free radicals with recurrent seizures (Xu and Fan 2022). In addition, it has been reported that in epilepsy, prolonged and recurrent seizures may cause lipid peroxidation and mitochondrial dysfunction in peripheral organs such as the liver and kidney, which are sensitive to oxidative stress (Dilliogluğil et al. 2010).

PTZ is a widely used agent to induce epilepsy-like tonic and clonic seizures in experimental animals (Goudarzi et al. 2020). In experimental studies using the PTZ model, it has been reported to increase oxidative stress in the brain (Li et al. 2021).

The liver and kidney are the detoxification organs of the organism (Ulusu et al. 2020). They are tissues that protect the body from environmental toxins and various xenobiotics, including drugs. Therefore, they are organs sensitive to oxidative stress. The mechanism of epilepsy disease is not fully understood. However, the diagnosis of acute liver failure, which develops as a complication of grand mal seizures, is determined by the evaluation of neurological disorders and liver damage markers together. In addition, hypoxia and steatosis-related factors that may develop due to recurrent seizures in peripheral organs are also considered (Dilliogluğil et al. 2010; Rodrigues et al. 2013).

ART, which is obtained from the plant Artemisia annua, is an agent used in the treatment of malaria worldwide (Dehkordi et al. 2019). It has also been shown to have anti-inflammatory, antibacterial, and anticancer effects. It has been reported that ART has an effect on the GABA receptor, which plays an important role in epilepsy, and is an NMDA receptor antagonist (Dehkordi et al. 2019; Kim et al. 2014; Kiss et al. 2021).

In this context, in this study, it was aimed to investigate the protective potential of ART in liver and kidney tissue against tissue damage that may occur due to oxidative stress that may occur in mice induced by PTZ.

MATERIAL AND METHODS

This study was approved by Van Yuzuncu Yil University (Turkey) Animal Researches Local Ethics Committee (YUHADYEK-28/07/2022-2022/07-14).

Animals and Experimental Design

In the experimental study, 42 Swiss albino mice were used. Mice were given access to standard pellet chow and water. mice were maintained on a 12-hour light/dark cycle. Afterward, the experimental animals were randomly divided into six groups of seven animals each. The first group was given saline (0.9% NaCl ip), and the second group was given isotonic dissolved PTZ (35 mg/kg, ip). The third group was given the reference drug valproate (VPA) (100 mg/kg, ip). Artemisinin in different doses of 30, 60 and 120 mg/kg was administered to the fourth, fifth and sixth groups. The last four groups (Groups 3,4,5,6) were injected with PTZ (35 mg/kg, ip) 30 minutes later.

Seizure Model

The PTZ-kindling epilepsy model was carried out with minor modifications to the method used by Ilhan et al. (Ilhan et al., 2005) Mice received a total of 11 injections of PTZ (35 mg/kg) every other day for 24 days. One hour before PTZ-treated, different doses of ART and VPA were administered. Mice were then observed for 30 minutes for tonic and clonic seizures. In addition, on the 26th day of this study, PTZ (75 mg/kg ip) dose was administered to the test groups (PTZ, VPA, ART). This dose is convulsion (clonic and tonic), status epilepticus and lethality dose (Ilhan et al. 2005; Kiasalari et al. 2013). Following PTZ injection, for 30 minutes, the presence of seizures and lethality were evaluated, recorded and scored.

Obtaining Homogenate from Liver and Kidney Tissue Samples

Mice were sacrificed, and tissues were removed and washed with isotonic saline. Phosphate buffer (pH 7.4, 1.8 mL 50 mM) was added to liver and kidney tissues (200 mg). Homogenization was achieved with a homogenator device (Ultra Turrax-T25). The obtained homogenate was centrifuged at 10000 rpm for 30 minutes. The supernatants were then stored at -80 °C until the day of the study. Protein concentrations of tissues were determined by the method of Lowry et al. (1951).

TOS Measurement of Liver and Kidney Tissue

TOS level was measured in liver and kidney tissue homogenates using a commercial kit (Rel Assay Kit Diagnostics, Turkey). Tissue TOS results were expressed as μmol H_2O_2 equivalent/gr protein (Erel 2005).

TAS Measurement of Liver and Kidney Tissue

TAS levels in liver and kidney tissue homogenates were measured using a commercial kit (Rel Assay Kit Diagnostics, Turkey). Tissue TAS results were expressed as mmol Trolox equivalent/gr protein (Erel 2004).

OSI (Oxidative stress index) Measurement

OSI is calculated as a percentage of the ratio of TOS to TAS. To find the OSI values, the unit of TAS is converted to μmol/gr protein. OSI levels were calculated according to the formula expressed below (Cikman et al. 2014).

OSI = (TOS, μ mol H_2O_2 equivalent/gr protein) / (TAS, μ mol Trolox equivalent/gr protein)/100)

Statistical Analysis

Descriptive statistics were obtained for the groups used in the study. Data were expressed as Mean ± SEM. A one-way ANOVA analysis of variance was used to compare the groups, followed by the Tukey post hoc test. SPSS (IBM SPSS for Windows, ver.24) program was used for the calculations.

RESULTS

In this study, the effects of artemisinin on the liver and kidney tissue in mice treated with PTZ were evaluated. The liver tissue TOS level of the PTZ group was found to be higher than all other groups ($p<0.05$). The liver tissue TOS levels of the control (C) and ART-120 groups were similar ($P>0.05$), and liver TOS levels were found to be significantly lower when these two groups were compared with the PTZ, VPA, ART-30 and ART-60 groups ($p<0.05$). When the liver TOS level of the VPA group was compared with the ART-30 and ART-60 groups, there was no statistical significance ($p>0.05$). The liver tissue results are

shown in figure 1. TOS levels in kidney tissue were high in PTZ and ART-120 groups (p<0.05). Group C kidney tissue TOS level was lower than all other groups (p<0.05). Among the ART groups, the lowest TOS level was found in the ART-30 dose (p<0.05). The kidney tissue results are shown in figure 2. TAS levels of liver tissue are shown in figure 1. The liver TAS level of the PTZ group was found to be lower than the other groups (p<0.05). The ART-120 group was found to be significantly higher than the other groups (p<0.05). The liver TAS level of group C was similar to the ART-30 group, significantly higher when compared to the PTZ, VPA and ART-60 groups, and significantly lower compared to the ART-120 group (p<0.05). Kidney tissue TAS levels of PTZ, ART-30 and ART-120 groups were found to be low (p<0.05). TAS level of kidney tissue in the VPA group was found to be significantly higher than in all other groups. While the kidney tissue TAS level of the control group was significantly lower than the VPA group, it was found to be significantly higher than the PTZ, ART-30, ART-60 and ART-120 groups (p<0.05, Figure 2).

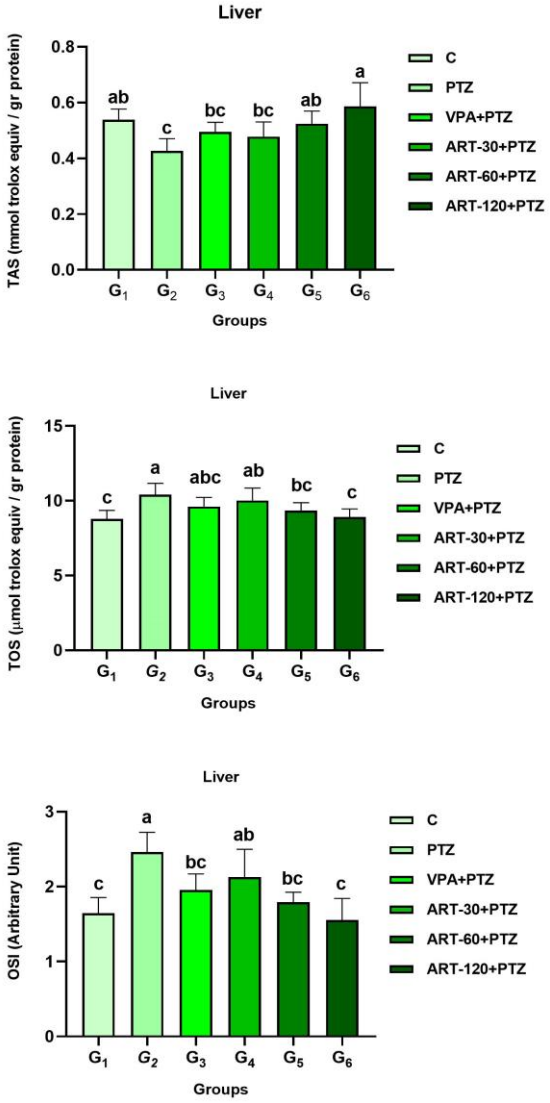

Figure 1. Comparison of the values of parameters measured in Liver homogenates. *Different letters in the same column represent statistical significance (p<0.05). C: Control/saline, PTZ: pentylenetetrazol, VPA: Valproate, ART: artemisinin, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index Values are mean ± SD. n = 7 per group;

Liver tissue OSI value was higher in the PTZ group compared to all other groups. The lowest OSI value was found in the ART120 group and this value was found to be significantly lower when compared to the groups (p<0.05). The OSI value of group C was found to be significantly higher than the ART120 group and significantly lower than the PTZ, VAR, ART30 and ART60 groups (p<0.05, Figure 1). In kidney tissue, group C OSI value was found to be significantly lower when compared to all other groups. PTZ group OSI value was lower than all groups (p<0.05, Figure 2).

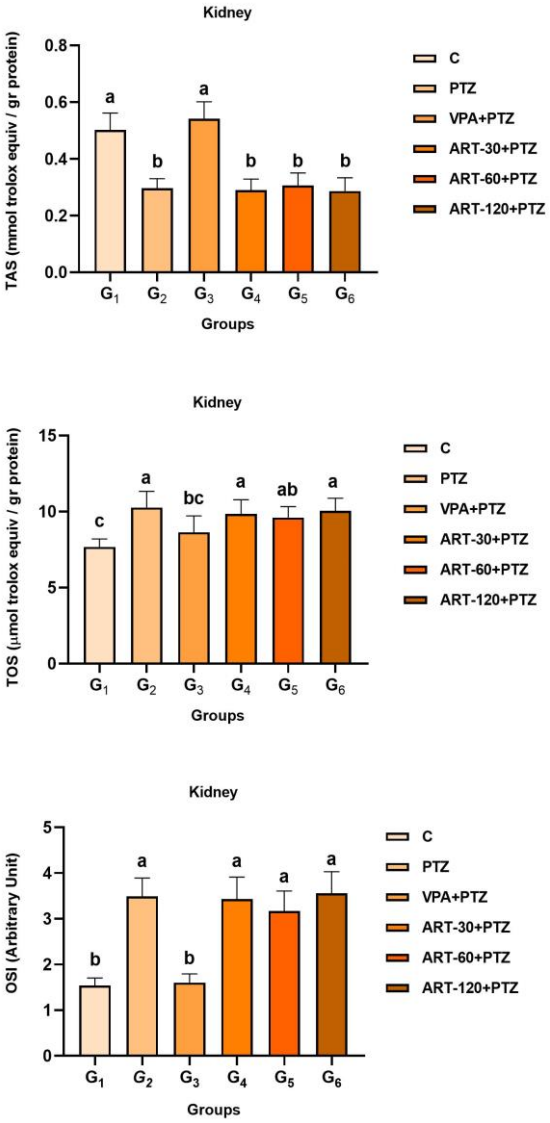

Figure 2. Comparison of the values of parameters measured in kidney homogenates. *Different letters in the same column represent statistical significance (p<0.05). C: Control/saline, PTZ: pentylenetetrazol, VPA: Valproate, ART: artemisinin, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index Values are mean ± SD. n = 7 per group;

DISCUSSION AND CONCLUSION

Oxidative stress (OS) may occur due to an excessive increase in free radicals in the body or insufficiency of the antioxidant defense system (Buyukuslu and Yigitbasi 2015). OS causes DNA damage and lipid peroxidation in the cell. This may contribute to the formation of tissue

damage (Bulduk 2022; Kurt et al. 2022). OS has been suggested to trigger the occurrence of various diseases. One of these diseases is epilepsy. Researchers reported that OS increases epileptic seizures and decreases the antioxidant defense system. Also, free radical production can increase recurrent seizures (Akbas et al. 2005). These recurrent seizures can lead to lipid peroxidation and oxidant/antioxidant imbalance in hepatocyte cells in the liver. In addition, it may cause fulminant liver failure, one of the complications of epilepsy (Rodrigues et al. 2013). It has also been reported that long-term use of antiepileptic drugs may cause hepatotoxicity by increasing liver enzyme levels (Cengiz et al. 2000; Ichai et al. 2003). Experiment results found that TOS increased and consumed TAS in PTZ-administrated groups. It was observed that ART increased the TAS level with increasing doses. In particular, the high dose of ART was better than the control and reference drug groups. The data of this study are compatible with previous studies (Akbas et al. 2005; Dillioglugil et al. 2010; Obay et al. 2008; Rodrigues et al. 2013; Sudha et al. 2001). Researchers show in their studies that epilepsy disease can trigger stress in the liver. In a previous study, they suggested that liver damage may be caused by ischemia and hypoxia due to epileptic seizures (Decell et al. 1994). ART affected the OS stress parameters in liver tissue. It has been reported that ART suppresses apoptosis induced by anesthetic drugs (Xu et al. 2017). It has also been suggested that ART has a strong antioxidant capacity against OS that can be caused by free radicals (Ahmed-Laloui et al. 2022; Egwu et al. 2022). They hypothesized that oxidative stress in the liver in experimental epileptic seizures would be due to the activation of glutamate receptors (Akbas et al. 2005). Our results suggest that the effect of ART in the liver is either by reducing lipid peroxidation in hepatocyte cells or by inhibiting glutamate receptor activation.

Armağan et al. (2008) reported that PTZ administration increased lipid peroxidation and decreased antioxidant enzymes (SOD, CAT) in the kidney. Moreover, it has been reported that rats treated with PTZ have increased urea, creatinine, uric acid and cystatin c levels. In the same study, kidney DNA fragmentation and MDA levels increased with PTZ. They also suggested that total antioxidant capacity decreased (Tousson et al. 2019). In their study conducted by different research groups, they reported that PTZ administration increased lipid peroxidation in the kidney, but there was no change in endogenous antioxidant levels (Kapucu et al. 2021; Uma Devi et al. 2006). As a matter of fact, in our study, it was determined that TOS levels increased and TAS levels decreased in the kidneys of mice treated with PTZ. Our results were similar to the literature. In recent studies, it has been hypothesized that PTZ administration causes OS by activating glutamate receptors. It has been reported that these receptors may be the source of OS occurring in peripheral organs due to seizures (Kapucu et al. 2021; Lüttjohann et al. 2009). ART pretreatment did not significantly improve total oxidant/antioxidant levels in the kidneys of PTZ-treated mice. The moderate dose of ART showed a modest increase in antioxidant levels. It has been reported that ART has curative effects in studies on kidney disorders (Xia et al. 2020). It has been reported that ART used in the treatment of lupus nephritis improves the creatinine clearance rate (Lu 2002). It has been reported that ART used in the treatment of lupus nephritis improves the creatinine clearance rate. They also claimed to improve proteinuria and glomerular permeability (Jin et al. 2009). In light of the literature, it is seen that ART and its derivatives have therapeutic effects on kidney tissue. However, in this study, it was determined that there was no significant curative effect against OS stress caused by PTZ in kidney tissue.

It was determined that PTZ increased the TOS level and decreased the TAS level in the liver and kidney tissues of the mice. ART pretreatment showed antioxidant properties by reducing oxidative stress in the liver. ART was less effective in kidney tissue. Although ART is currently used effectively against malaria, it may be useful in reducing oxidative stress in peripheral tissues that can be caused by epileptic seizures. Further experimental and clinical studies are needed to understand the mechanism of the beneficial effects of ART.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

This study was approved by Van Yuzuncu Yil University (Turkey) Animal Researches Local Ethics Committee (YUHADYEK-28/07/2022-2022/07-14).

AUTHOR CONTRIBUTIONS

Idea / Concept: YK
Supervision / Consultancy: YK
Data Collection and / or Processing: YK
Analysis and / or Interpretation: YK
Writing the Article: YK
Critical Review: YK

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