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RESEARCH ARTICLE

Effect of Alpha Lipoic Acid on Polychlorinated Biphenyl (Aroclor 1254)-Induced Nephrotoxicity in Rats

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ABSTRACT

The present study aimed to determine the morphological changes in rat kidneys exposed to PCB (Aroclor 1254) and to reveal the effects of ALA on those changes. Rats were randomly divided into four groups: Control, ALA, PCB and ALA+PCB. The applications of ALA and PCB continued for thirty days. At the end of the experiment, the tissue samples were taken from the animals. Crossmon's triple staining method was applied to kidney sections for histopathological and histomorphometric examinations. In addition, the periodic acid-Schiff (PAS) reaction was examined in tissue sections. Histopathologically, it was determined that glomerular degeneration and connective tissue densities in the kidney significantly increased in PCB group compared to Control group. Besides, it was found that glomerular degeneration intensity significantly decreased in ALA+PCB group compared to PCB group. It was detected that the diameters of the renal corpuscle, proximal tubule, ascending limb of Henle's loop and collecting duct in the kidney significantly decreased in PCB group compared to Control group. However, these parameters significantly increased in ALA+PCB group compared to PCB group. While the density of glomerular mesangial matrix and glycogen density in proximal tubule epithelium significantly increased in PCB group compared to Control group, these parameters significantly decreased in ALA+PCB group compared to PCB group. As a result, PCB may adversely affect renal functions by causing disorders in kidney histology. In addition, the use of ALA against PCB exposure may contribute to the preservation of kidney tissue.

Keywords: Alpha lipoic acid, Aroclor 1254, Histology, Kidney, Rat

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Sıçanlarda Poliklorlu Bifenil (Aroklor 1254) ile Oluşturulan Nefrotoksisite Üzerine Alfa Lipoik Asitin Etkisi

ÖZ

Sunulan çalışma, PCB (Aroklor 1254)'ye maruz bırakılan sıçanların böbreklerindeki morfolojik değişikliklerin belirlenmesini ve bu değişiklikler üzerine ALA'nın etkilerinin ortaya çıkarılmasını amaçladı. Sıçanlar kontrol, ALA, PCB ve ALA+PCB olmak üzere rastgele dört gruba ayrıldı. ALA ve PCB uygulamaları otuz gün boyunca devam etti. Deney sonunda hayvanlardan doku örnekleri alındı. Histopatolojik ve histomorfometrik incelemeler için böbrek kesitlerine Crossmon'un üçlü boyama yöntemi uygulandı. Ayrıca doku kesitlerinde periyodik asit-Schiff (PAS) reaksiyonu incelendi. Histopatolojik açıdan, böbrekte glomerüler dejenerasyon ile bağ doku yoğunluklarının kontrol grubuna göre PCB grubunda anlamlı bir şekilde arttığı tespit edildi. Bununla birlikte, glomerüler dejenerasyon yoğunluğunun PCB grubuna göre ALA+PCB grubunda anlamlı bir şekilde azaldığı saptandı. Böbrekte *renal korpüskül,* proksimal tübül, çıkan henle ve toplayıcı kanal çaplarının kontrol grubuna göre PCB grubunda anlamlı bir şekilde azaldığı belirlendi. Ancak bu parametreler PCB grubuna göre ALA+PCB grubunda anlamlı bir şekilde arttı. Böbrekte glomerüler mezengial matriks yoğunluğu ile proksimal tübül epitelindeki glikojen yoğunluğu kontrol grubuna göre PCB grubunda anlamlı bir şekilde artarken, bu parametreler PCB grubuna göre ALA+PCB grubunda anlamlı bir şekilde azaldı. Sonuç olarak, PCB böbrek histolojisinde bozukluklara neden olarak renal fonksiyonları olumsuz etkileyebilir. Bununla birlikte, PCB maruziyetine karşı ALA kullanımı böbrek dokusunun korunmasına katkı sağlayabilir.

Anahtar Kelimeler: Alfa lipoik asit, Aroklor 1254, Böbrek, Histoloji, Sıçan

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are compounds that are found in many industrial products and cause environmental pollution (Aydin et al. 2007). PCBs are frequently used in printing ink, pesticides, adhesives, fluorescent lamps, machine oils and dental filling materials (Lindell 2012). Exposure to PCBs occurs through their transfer to air, soil and water systems during production or usage (Bruner-Tran and Osteen 2010). The exposure through digestion usually carries out with the consumption of contaminated fish (Seyran and Erisir 2008). It has also been determined that PCBs pass to the fetus via the placenta and to the newborn via milk (Lilienthal et al. 2000). PCBs tend to accumulate in adipose tissue due to their lipophilic properties (Carpenter 2005). This is a clear indication that the health of people exposed to PCBs is at risk for a long time (Seyran and Erisir 2008). Besides, it has been reported that PCBs have carcinogenic, immunosuppressive, disrupting the functions of the genital system and hormonal system, neurotoxic and hepatotoxic effects (Oskam et al. 2004; Hsu et al. 2007; Murugesan et al. 2007).

PCB exposure reduces kidney weight (Banudevi et al. 2006) and significantly increases serum urea, creatinine and uric acid levels (Kutlu et al. 2007). In addition, it has been found that PCBs cause histopathological lesions such as apoptosis (Ghosh et al. 2010) enlargement of tubular cells, vacuole formation, necrosis (Raja et al. 2019), inflammation (Sula et al. 2020), edema, congestion (Lu et al. 2009) and corpuscular atrophy in kidney tissue (Kutlu et al. 2007). On the other hand, PCB decreases the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) in kidney tissue, in contrast, increases lipid peroxidation, hydrogen peroxide and hydroxyl radical levels (Banudevi et al. 2006).

Alpha lipoic acid (ALA) is a fatty acid with strong antioxidant activity. It is found in foods such as red meat, broccoli, spinach, tomatoes and peas (Karaca and Sozbilir 2007). Also, it is synthesized by lipoic acid synthase in mitochondria intracellularly (Padmalayam et al. 2009). The antioxidant effect of ALA is not only through its suppression of free radical formation but also indirectly through the reuse of other cellular antioxidants (Guimaraes et al. 2007). Due to the antioxidant role of ALA, its positive effects have been observed in various pathological conditions such as diabetes, atherosclerosis, neuron degeneration, multiple sclerosis and joint diseases (Singh and Jialal 2008).

Results of studies that use different experimental models demonstrated that ALA decreased the plasma urea level (Pradhan et al. 2013) and the degree of polyuria by increasing the urine osmolality (Bae et al. 2009). Similarly, ALA significantly reduced glomerular and vascular damage, leukocyte infiltration (Mervaala et al. 2003), tubulointerstitial fibrosis (Bhatti et al. 2005), basement membrane thickening (Winiarska et al. 2008), tubular dilatation, mesangial enlargement (Oksala et al. 2007), vacuolization (El-Sawalhi and Raafat 2007), collagen deposition (Grdović et al. 2021), as well as the rates of congestion and apoptosis in the kidney tissue (Cakir et al. 2015). In addition, while the expression of Bcl-2 increased, the Bax (Gultekin et al. 2021) and caspase 3 expressions, which play a role in the apoptotic process decreased with the use of ALA in the kidney tissue (Oktan et al. 2021). On the other hand, ALA caused a significant increase in GPx, CAT and GSH levels, as well as a decrease in MPO activity and MDA level in kidney tissue (Jamor et al. 2019).

The present study aimed to examine the morphological changes in the kidneys of rats with PCB toxicity and to reveal the effects of ALA on those changes.

MATERIAL and METHODS

Animals

Thirty-two 3-month-old Wistar Albino adult male rats were used in the study. The rats were obtained from Aydin Adnan Menderes University Experimental Animals Unit. The rats had ad libitum to water and food in a controlled environment at a temperature of 22±1 °C, following a 12-hour light/dark cycle. The study was carried out with the permission of Aydin Adnan Menderes University Animal Experiments Local Ethics Committee (No: 64583101/2021/180).

Experimental Design

The rats were randomly divided into four groups; Control (n=8), ALA (n=8), PCB (n=8) and ALA+PCB (n=8). The control group received corn oil at a dose of 2 $ml.kg^{-1}$ by oral gavage for 30 days. ALA group was administered 25 $mg.kg^{-1}$ of ALA (Sigma T5625, Schnelldorf, Germany) dissolving in 2 $ml.kg^{-1}$ corn oil by oral gavage for 30 days. The PCB group was supplemented with 5 $mg.kg^{-1}$ of PCB (Sigma 48586, Schnelldorf, Germany) dissolved in 2 $ml.kg^{-1}$ corn oil by oral gavage for 30 days. ALA+PCB group was treated with 25 $mg.kg^{-1}$ of ALA dissolved in 1 $ml.kg^{-1}$ corn oil and one hour later 5 $mg.kg^{-1}$ of PCB dissolved in 1 $ml.kg^{-1}$ corn oil was applied by oral gavage for 30 days (Gules and Eren 2016).

Sample Collection and Preparation

At the end of the experiment, the rats were anesthetized by intraperitoneal (ip) administration of 80 $mg.kg^{-1}$ of ketamine (Pfizer, Istanbul, Turkey) and 20 $mg.kg^{-1}$ of xylazine (Bayer, Istanbul, Turkey). Then, a midline incision was made in the abdominal wall of the animals that were killed by cervical

dislocation. Kidney tissues were removed from the abdomen. The right kidney tissue was fixed in 10% buffered neutral formalin solution for 24 hours. Following standard histological processing, the tissue samples were embedded in paraffin and serial sections were obtained at intervals of 100 μm, each being 5-6 μm thick.

Histomorphometric Analysis

Histomorphometric examinations were carried out on tissue sections stained with Crossmon's triple staining method. The diameters of the renal corpuscle, proximal tubule, distal tubule, ascending limb of Henle's loop and collecting duct were measured in the sections. Histomorphometric measurements were executed by making five different measurements on five sections of each animal at 40x magnification. All microscopic processes were made by a light microscope (SOIF BK5000-TR/L, Denmark) equipped with an image analysis system (MShot Digital Imaging System, China). In addition, photographs were taken with a camera (MShot MD 50, China) from the necessary parts of the sections.

Histopathological and Histochemical Analyzes

The kidney sections were stained with Crossmon's triple staining method for histopathological evaluation. Tissue sections were examined in terms of glomerular degeneration and connective tissue densities. In histopathological examination, scoring was made for each parameter in three sections of each animal. In the histochemical examination, the sections were stained with the Periodic acid-Schiff (PAS) staining method to reveal neutral mucosubstance and glycogen. As a result of the staining, glomerular mesangial matrix density and glycogen density in the proximal tubule epithelium were evaluated. The densities of histochemical parameters were determined in 10 different structures of three sections belonging to each animal by scoring for each parameter. The sections' histopathological and histochemical evaluations were made semi-quantitatively and scored between 0-3 (0 = none; 1 = weak; 2 = moderate; 3 = severe)(Sirca et al. 2012).

Statistical Analysis

Data were analysed with SPSS 17.00 package program. Whether there was a difference between the groups was determined by one-way analysis of variance (ANOVA). The post hoc Duncan test was applied to detect which group or groups caused the difference. Data are presented as mean ± Standard Error. Values with differences of $p<0.01$ (**) and $p<0.001$ (***) were considered statistically significant (Gules et al. 2022).

RESULTS

In the histomorphometric analysis performed on kidney tissue; the diameters of the renal corpuscle, proximal tubule, distal tubule, ascending limb of Henle's loop and collecting duct were measured in the Control and Experimental groups. The data are shown in Table 1. It was determined that the diameters of the renal corpuscle, proximal tubule, ascending limb of Henle's loop and collecting duct in the kidney significantly decreased in the PCB group compared to the Control group. At the same time, they significantly increased in the ALA+PCB group compared to the PCB group ($p<0.001$). There was no significant difference between the groups in terms of distal tubule diameter (Figure 1 and Figure 2).

In the histopathological analysis of kidney tissues, the Control and Experimental groups were examined in terms of glomerular degeneration and connective tissue densities. The obtained data are given in Table 2. It was detected that glomerular degeneration and connective tissue densities significantly increased in the PCB group compared to the Control group ($p<0.001$). Also, it was found that glomerular degeneration significantly decreased in the ALA+PCB group compared to the PCB group. However, there was no significant difference in connective tissue density between PCB and ALA+PCB groups (Figure 3).

In the histochemical analysis carried out on kidney tissue, the Control and Experimental groups were examined in terms of glomerular mesangial matrix density and glycogen density in the proximal tubule epithelium. The obtained data are shown in Table 3. It was determined that the PAS-positive reaction densities in the glomerular mesangial matrix and proximal tubule epithelium significantly increased in the PCB group compared to the Control group, and they significantly decreased in the ALA+PCB group compared to the PCB group ($p<0.01$; $p<0.001$) (Figure 4).

Table 1. Histomorphometric values that were obtained from the kidney tissues of Control and Experimental groups.

Groups	n	Diameter of renal corpuscle (μm)	Diameter of proximal tubule (μm)	Diameter of distal tubule (μm)	Diameter of ascending limb of Henle's loop (μm)	Diameter of collecting duct (μm)
Control	8	93.59±0.59^{a}	34.08±0.25^{a}	26.11±0.21	32.17±0.29^{a,b}	33.48±0.26^{a}
ALA	8	91.67±1.02^{a}	33.80±0.33^{a}	26.14±0.20	32.09±0.27^{a,b}	32.67±0.24^{a,b}
PCB	8	85.45±0.62^{b}	32.30±0.37^{b}	26.45±0.20	29.75±0.21^{c}	30.27±0.22^{c}
ALA+PCB	8	93.01±0.62^{a}	34.02±0.21^{a}	26.94±0.39	31.48±0.20^{b}	31.93±0.23^{b}
P		***	***	NS	***	***

$^{a,b,c:}$ Different superscripts in the same column indicate the significant difference. NS: Non-significant, ***$p<0.001$

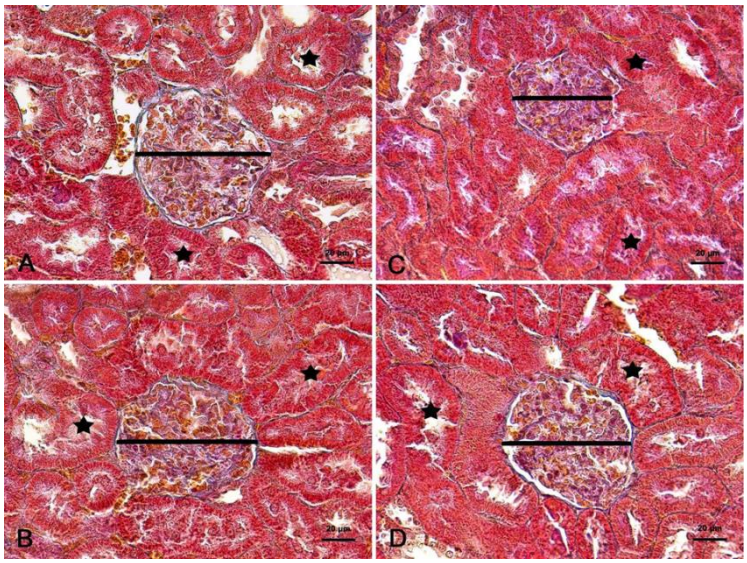

Figure 1: Microscopic appearances of of kidney tissues in Control (A), ALA (B), PCB (C) and ALA+PCB (D) groups. It is observed that the diameters of the renal corpuscle (black lines) and proximal tubule (stars) decreased in the PCB group compared to the Control group, and they increased in the ALA+PCB group compared to the PCB group. Crosmon's triple stain method. Bars: 20µm.

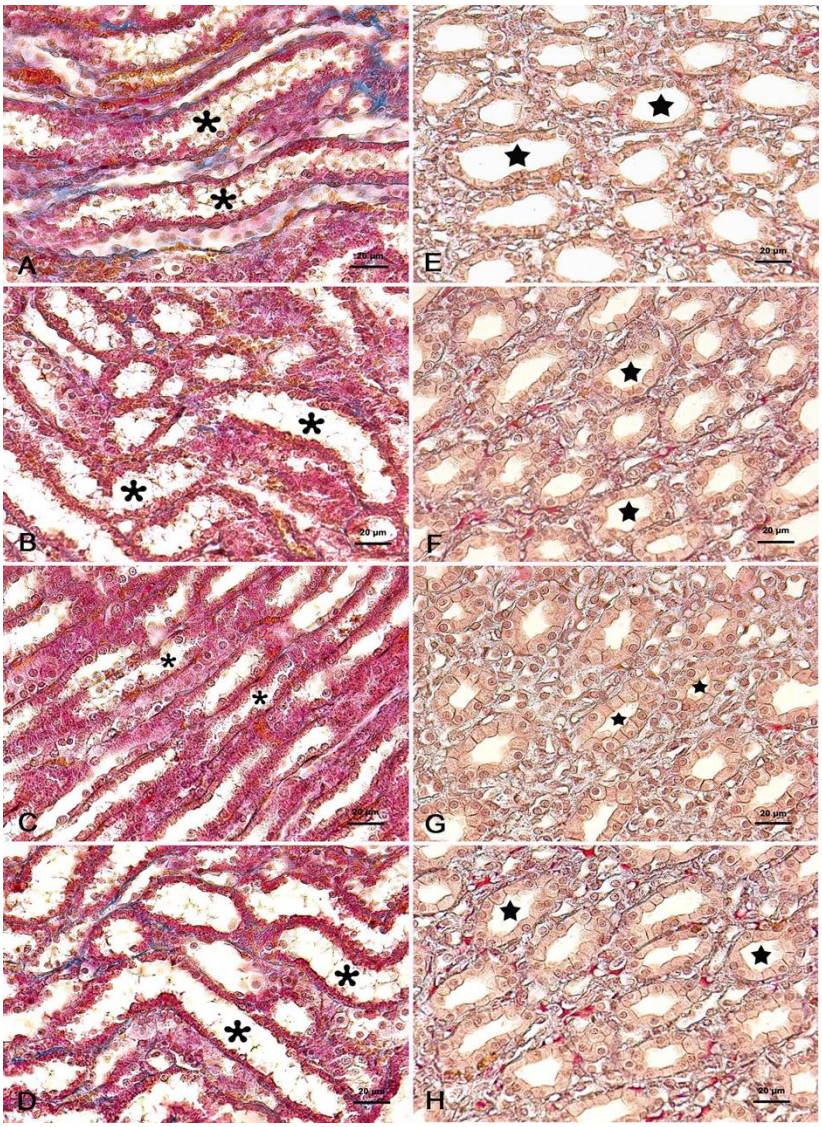

Figure 2: Microscopic views of kidney tissues in Control (A, E), ALA (B, F), PCB (C, G) and ALA+PCB (D, H) groups. It is seen that the diameters of the ascending limb of Henle's loop (asterisks) and collecting duct (stars) decreased in the PCB group compared to the Control group and they increased in the ALA+PCB group compared to the PCB group. Crosmon's triple stain method. Bars: 20µm.

Table 2. Density values of histopathological changes in kidney tissues of Control and Experimental groups.

Groups	n	Glomerular degeneration	Amount of connective tissue
Control	8	$0.10\pm0.04^{b,c}$	0.74 ± 0.04^{b}
ALA	8	$0.12\pm0.05^{b,c}$	0.66 ± 0.08^{b}
PCB	8	0.52 ± 0.05^{a}	1.14 ± 0.05^{a}
ALA+PCB	8	0.27 ± 0.03^{b}	1.17 ± 0.05^{a}
P		***	***

[a,b,c:] Different superscripts in the same column indicate the significant difference. ***p<0.001.

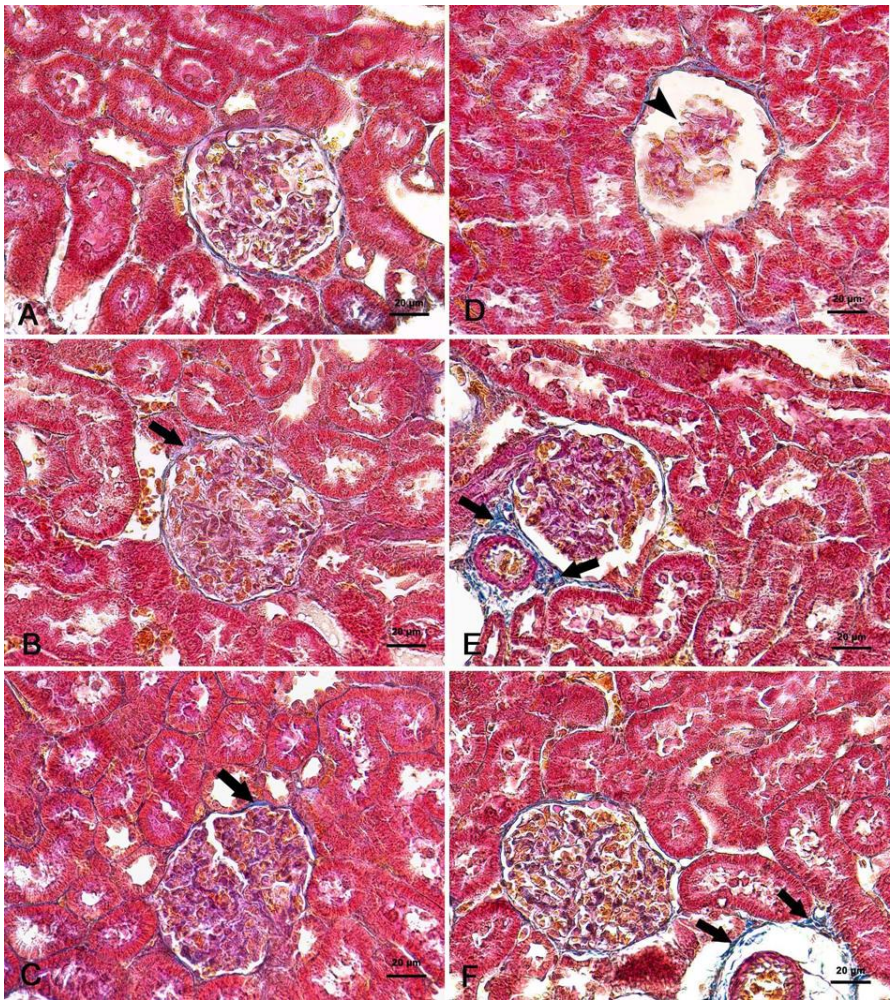

Figure 3: Microscopic appearances of kidney tissue sections in Control (A, B), ALA (C), PCB (D, E) and ALA+PCB (F) groups. It is observed that glomerular degeneration (arrowhead) and connective tissue (arrows) densities increased in the PCB group compared to the Control group, while glomerular degeneration decreased in the ALA+PCB group compared to the PCB group. Crosmon's triple staining method. Bars: 20µm.

Table 3. Density of glomerular mesangial matrix and glycogen density in proximal tubule epithelium in kidney tissues of Control and Experimental groups.

Groups	n	Density of glomerular mesangial matrix	Glycogen density in proximal tubule epithelium
Control	8	1.62 ± 0.03^{b}	1.53 ± 0.03^{b}
ALA	8	1.74 ± 0.05^{b}	1.61 ± 0.03^{b}
PCB	8	1.86 ± 0.03^{a}	1.78 ± 0.03^{a}
ALA+PCB	8	1.69 ± 0.03^{b}	1.50 ± 0.03^{b}
P		**	***

[a,b:] Different superscripts in the same column indicate the significant difference. **p<0.01, ***p<0.001.

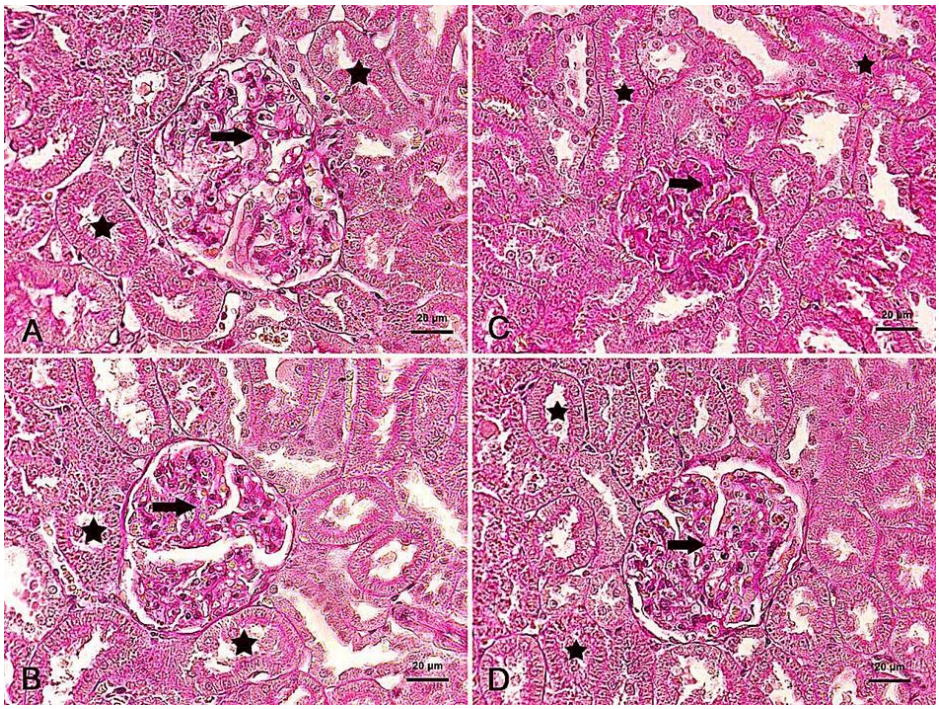

Figure 4: PAS-positive reaction in kidney tissues of Control (A), ALA (B), PCB (C) and ALA+PCB (D) groups. It is noticed that the PAS-positive reaction densities in the glomerular mesangial matrix (arrows) and proximal tubule epithelium (stars) increased in the PCB group compared to the Control group, and they decreased in the ALA+PCB group compared to the PCB group. Periodic acid-Schiff staining method. Bars: 20µm.

DISCUSSION

PCB (A1254) is a chemical that leads to environmental pollution and causes corpuscular atrophy in the rat kidney (Kutlu et al. 2007). Atrophy also has been observed in the tubular and glomerular cells in the kidneys of fishes living in PCB-contaminated water (Sula et al. 2020). In the present study, it was determined that the diameters of the renal corpuscle, proximal tubule, ascending limb of Henle's loop and collecting duct decreased in the PCB group compared to the Control group. These results suggest that PCB induces structural changes in the kidney and negatively affects nephron functions.

PCB exposure causes disintegration, loss of nuclear material, vacuole formation and necrosis in tubular cells in the mouse kidney (Raja et al. 2019). It has also been reported that edema in renal proximal tubule cells and congestion in the kidney medulla were observed in rats administered PCB (Lu et al. 2009). In the kidneys of fish living in PCB-contaminated water; peritubular fibrosis characterized by inflammation, glomerular necrosis, thickening of the Bowman capsule, hyaline degeneration in the interstitial tissue and fibroblast infiltration have been detected (Sula et al. 2020). On the other hand, it has been determined that faster apoptotic cell death occurred depending on the time when human kidney cells in the culture medium were exposed to PCB (Ghosh et al. 2010). When the histopathological data in our study was examined, it was noted that glomerular degeneration and connective tissue densities increased in the PCB group compared to the Control group. The findings in our study are similar to the literature, and it can be thought that PCB damages kidney tissue histology and thus it may play a role in the pathogenesis of kidney diseases.

There are very limited data on how PCB affects the PAS reaction in kidney tissue. In the study of Sonne et al. (2008), it was reported that PCB exposure caused PAS-positive glomerular mesangial accumulations and glomerular basement membrane thickening in the fox kidney. Similarly, the present study determined that the PAS-positive reaction densities in the glomerular mesangial matrix and proximal tubule epithelium increased in the PCB group compared to the Control group. Mesangial cell proliferation and increased PAS-positive reaction in the mesangial matrix are associated with glomerulosclerosis (Tomooka et al. 1992). Based on this data; it can considered that PCBs may contribute to the pathogenesis of glomerulosclerosis and trigger renal dysfunction.

ALA has a strong antioxidant effect which plays a role in protecting cells from the effects of oxidative stress. Studies showed that ALA significantly reduced the rate of glomerulosclerosis, tubulointerstitial fibrosis (Bhatti et al. 2005), tubular dilatation (Oksala et al. 2007), vacuolization, degeneration (Morakinyo et al. 2012), inflammation (Gultekin et al. 2021) and apoptosis in the kidney (Oktan et al. 2021). In addition, it has been demonstrated that ALA widened the narrowed Bowmann capsule cavity (Gultekin et al. 2021). In the current study, we found similar

results. The diameters of the renal corpuscle, proximal tubule, ascending limb of Henle's loop and collecting duct, which decreased as a result of PCB exposure, increased with the effect of ALA. Besides, it was observed that glomerular degeneration caused by PCBs was reduced by ALA application. Thus, it was demonstrated for the first time that ALA contributed to the preservation of histological structure in the kidney during PCB exposure. In contrast, ALA didn't affect the connective tissue density increased by PCB in the current study. The study of Wongmekiat et al. (2013) showed that ALA decreased the rate of fibrotic areas formed in the kidney in obstructive nephropathy. The reason for obtaining different results in our study may be due to differences such as the applied experimental model, application duration and application method. On the other hand; in our study, it was determined that the PAS-positive reaction densities which increased in the glomerular mesangial matrix and proximal tubule epithelium in the PCB group, decreased with the effect of ALA. Similarly; in the study by Bhatti et al. (2005), it was shown that the positive PAS reaction in the mesangial matrix in the kidneys of rats with experimental diabetes was alleviated by the effect of ALA. As mentioned above, increased PAS-positive reaction in the mesangial matrix are associated with glomerulosclerosis (Tomooka et al. 1992). Based on this information, ALA application against PCB toxicity may reduce the risk of glomerulosclerosis.

Oxidative stress stimulates the development of processes such as mutation, apoptosis, and aging in the cell and ultimately causes tissue damage (Valko et al. 2006). In the study of Banudevi et al. (2006), PCB exposure reduced the activities of antioxidant enzymes such as SOD, CAT, GPx and GR in kidney tissue. On the contrary, it has been determined that PCB triggered the formation of oxidative stress by significantly increasing lipid peroxidation, hydrogen peroxide and hydroxyl radical levels. Based on these data, it can be stated that the reason for the disorders in the kidney that caused due to PCB exposure in our study may be oxidative stress that was occurred by PCBs in the organism. On the other hand, ALA is a free radical-scavenging antioxidant and protects the organism from oxidative damage (Karaca and Sozbilir 2007). Studies have shown that ALA reduced malondialdehyde (MDA) levels through increased MPO activity in kidney tissue of rats with experimental ischemia reperfusion injury (El-Sawalhi and Raafat 2007) and diabetes (Jamor et al. 2019); while it significantly increased the decreased GPx, CAT and GSH levels. Accordingly, the reason for the improvement in kidney tissue disorders in our study may be that ALA protects cells by suppressing the oxidative stress triggered by PCBs.

CONCLUSION

In conclusion; PCB may negatively affect renal physiology by causing morphological changes in kidney tissue structure. Additionally, the use of ALA against PCB exposure may contribute to the protection of kidney histology. In addition, the findings obtained in our study were presented for the first time and they will serve as a reference for further studies in the future.

Conflict of interest: The author has no conflicts of interest to report.

Authors' Contributions: Conceptualization, Methodology, Research, Data curation, Visualization, Writing, Review and Editing were done by Mustafa Yıldız.

Ethical approval: This study was carried out at Aydin Adnan Menderes University Experiment Animals Application Center. The study was approved by Aydin Adnan Menderes University Animal Experiments Local Ethics Committee (ADUHADYEK Ref No: 6458310/2021/180).

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