

ORIGINAL ARTICLE

Medicine Science 2021;10(4):1122-7

Morphometric alteration induced by cyclophosphamide in rat kidney and protective efficacy of coenzyme Q10: A stereological study **Ahmad Yahyazadeh***Karabuk University, Faculty of Medicine, Department of Histology and Embryology, Karabuk, Turkey*

Received 27 March 2021; Accepted 17 May 2021

Available online 15.08.2021 with doi: 10.5455/medscience.2021.03.099

Copyright@Author(s) - Available online at www.medicinescience.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

**Abstract**

Cyclophosphamide (CD), as a chemotherapeutic drug, is used for the elimination of cancer cells, but its toxicity has led to health problems. We aimed to investigate the efficacy of coenzyme Q10 (C10) on kidney alterations caused by CD treatment in Wistar albino rats. Thirty-five adult male rats were assigned into five groups: control (CO), olive oil (OO), CD, C10, and CD+C10. All kidney tissues were analysed using the stereological and histopathological techniques. Stereological results revealed that the mean volumes of kidney, cortex, medulla, and glomerulus were significantly increased in the CD group than the CO group ($p < 0.05$). We also observed a significant decrease in the mean volume of proximal tubules in the CD group than the CO group ($p < 0.05$). In the CD+C10 group, the mean volumes of kidney, cortex, medulla, and glomerulus were significantly less when compared to the CD group ($p < 0.05$). In addition, there was a significant increase in the mean volume of proximal tubules in the CD+C10 group than the CD group ($p < 0.05$). Histopathological examination also revealed valuable results. Our findings showed that treatment with CD caused destructive change in the rat kidney tissues. Moreover, C10 administration significantly attenuated the toxic effect of CD on the kidneys.

Keywords: Coenzyme Q10, cyclophosphamide, kidney, rat**Introduction**

Cancer as a growing health problem can cause concern, difficulties, and ultimately death in individuals throughout the world. Chemotherapy is a treatment method, which is used to stop the growth and proliferation of target cancer cells [1]. The toxic effect of chemotherapeutic drugs on healthy organs has raised public health concerns. It has been reported that the use of high-dose cytotoxic drugs can also increase the drug side effect in cancer patients [2].

Cyclophosphamide (CD) is an alkylating agent that is commonly used as a chemotherapeutic drug in cancer treatment. CD is activated in the liver to 4-hydroxy-cyclophosphamide, which is isomerized to aldophosphamide. Acrolein and phosphoramidate mustard, as active metabolites are then produced from the conversion of aldophosphamide [3]. While the antineoplastic effects of CD are related to phosphoramidate mustard, its toxic

side effects are derived from acrolein. There are various factors such as age and dose that are involved in the side effect of CD [4]. Also, damage to healthy tissues vary from person to person. The main reason for the harmful impact of CD is that it is used in high doses. There has been documented a relationship between administration of CD and induction of toxicity in various vital system [5]. Moreover, cardiotoxicity has been reported as a serious complication induced by CD that is extensively utilized in treatment of cancer [6]. Urinary system is also vulnerable to change induced by chemotherapeutic agents [7]. Haemorrhagic cystitis is one of the most important complications seen after treatment with CD [8]. Side effect of CD can also be associated with a disorder in the kidney tissues, which is a major part of the urinary system.

Coenzyme Q10 (C10) is a fat-soluble compound found in every cell [9]. This vitamin-like substance plays a substantial role in production of cellular energy. The amount of C10 synthesized in humans and all animals is age- and health problem-dependent. Furthermore, C10 is found in the highest concentrations in the kidney, liver, pancreas, and heart. Sangsefidi et al. [10] documented the antioxidative activity of C10, which attenuates oxidative stress via scavenging free radicals and improving endogenous antioxidant defence. C10 has been reported to have anti-inflammatory

***Corresponding Author:** Ahmad Yahyazadeh, Karabuk University, Faculty of Medicine, Department of Histology and Embryology, Karabuk, Turkey
E-mail: yahyazadeh.ahmad@karabuk.edu.tr

properties through the elevation of anti-inflammatory cytokines [8].

Recently, researchers have been interested in the prevention of side effects of many chemotherapeutic drugs on vital organs, but the studies that conduct on kidneys are limited. The main purpose of this study was to investigate the therapeutic potential of C10 on morphometric changes of kidney tissues in rats exposed to CD by means of the stereological methods.

Materials and Methods

Thirty-five adult male Wistar albino rats (250-300 g body weight and 10-12-weeks old) were used in the present study. Ethical approval of this study was granted by Laboratory Animal Ethics Committee of Gazi University (26.08.2020, E.19891). All rats were obtained from the Experimental Animal Research and Application Centre of Pharmacy Faculty of Gazi University, Ankara. Animals were housed in plastic cages under 12-12 h light/dark cycle at a temperature of $22 \pm 2^\circ\text{C}$ and humidity of $50 \pm 5\%$ with free access to food and tap water. The experimental period for this study was 10 days. After rats were assigned into five groups, the experimental procedure was carried out as follow:

1. Control (CO) group (n = 7): Rats was treated with no substance during the experimental period.
2. Olive oil (OO) group (n = 7): Rats were administered 2.5 ml OO orally for 10-day experimental period. We used olive oil as a solvent for C10, and 4 mg/kg C10 was dissolved in 1 ml OO solution.
3. Cyclophosphamide (CD) group (n = 7): Rats were administered a single intraperitoneal injection of 150 mg/kg CD on the first day of the experiment [11].
4. Coenzyme Q10 (C10) group (n = 7): Rats were intraperitoneally administered 10 mg/kg/day C10 for 10 days [12].
5. Cyclophosphamide + coenzyme Q10 (CD+C10) group (n = 7): Rats were given a single intraperitoneal injection of 150 mg/kg CD on the first day of the experiment. In addition, animals were intraperitoneally administered 10 mg/kg/day C10 for 10 days.

Finally, we anesthetized the animals intraperitoneally by giving 80 mg/kg ketamine and 5 mg/kg xylazine. Rats was then perfused intracardiacally with fixative solution. Briefly, the right atrium was cut to allow drainage blood, then perfused with physiological saline until blood was flush from the cardiovascular system. Subsequently, the appropriate amount of fixative solution was given to the left ventricle with a certain pressure. Lastly, right Kidneys were removed immediately for stereological analysis.

Histology

We utilized 10% formalin for fixation of the kidney tissues, followed by routine tissue processing, including dehydration, impregnation, embedding, and blocking [13, 14]. Kidney samples were cut into 7- μm -thick sections using the systematic random sampling method, then sections were stained with haematoxylin-eosin [15]. Subsequently, micrographs were obtained from each section to examine the histopathological and morphometric

parameters of the kidney tissues.

Stereology

The mean volumes of the regions of interest in the kidney tissues were estimated using the Cavalieri technique [16]. A pilot study was determined that the point-counting grid was appropriate for the present research. Briefly, the kidneys of all groups were cut into consecutive sections at 1/240 intervals. All sections were photographed and transferred to a private computer. An appropriate grid was overlaid on micrographs, and then the number of points hitting the interest regions of kidney was counted. Lastly, the area of kidneys was estimated as:

$$\text{Area}(A) = a(p) \times \sum P$$

Where, “a(p)” is the area of point interval, and “ $\sum P$ ” is the point number counted in all sections. The total volume of interest regions was calculated as:

$$\text{Volume}(V) = t \times A$$

Where, “t” is the sum of section thickness and interval, and “A” is the total area of the interest region.

The CE and CV confirmed the sufficient point number counted in each animal and group, respectively.

Statistical analysis

SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data analysis were performed using One-Way ANOVA and the Tukey's post hoc test. Mean $\pm$ standard deviation (SD) was used for result expression. P value was statistically significant at less than 0.05.

Results

Stereology

The mean kidney volumes of all groups are shown in Figure 1. Volumetric findings showed that the mean volume of kidney was significantly higher in the CD group than the CO group ($p < 0.00$). To the contrary, there was a significant reduction in the mean volume of kidney in the CD+C10 group when compared with the CD group ($p < 0.01$). No difference was detected in the OO, C10, and CD+C10 groups than the CO group.

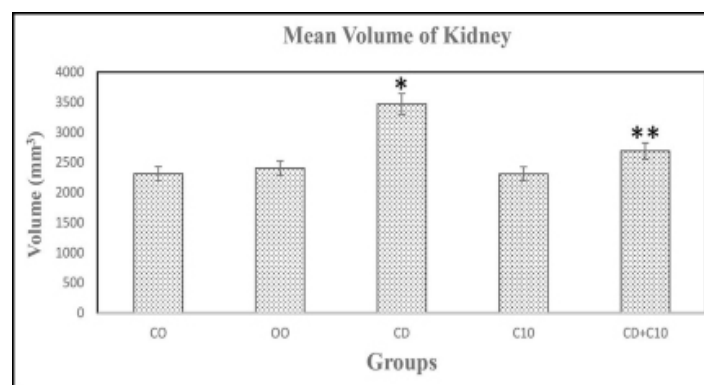


Figure 1. Graphs of the mean volumes of kidney in the CO, OO, CD, C10, and CD+C10 groups. *, a significant difference between the CD and CO groups; **, a significant difference between the CD+C10 and CD groups. CO, control; OO, olive oil; CD, cyclophosphamide; C10, coenzyme Q10; CD+C10, cyclophosphamide + coenzyme Q10

The mean cortex volumes of all groups are shown in Figure 2. The mean volume of cortex was significantly higher in the CD group when compared with the CO group ($p < 0.00$). Our volumetric findings also indicated a significant decrease in the mean volume of cortex in the CD+C10 than the CD group ($p < 0.01$). No difference was detected between the OO, C10, or CD+C10 groups and the CO group.

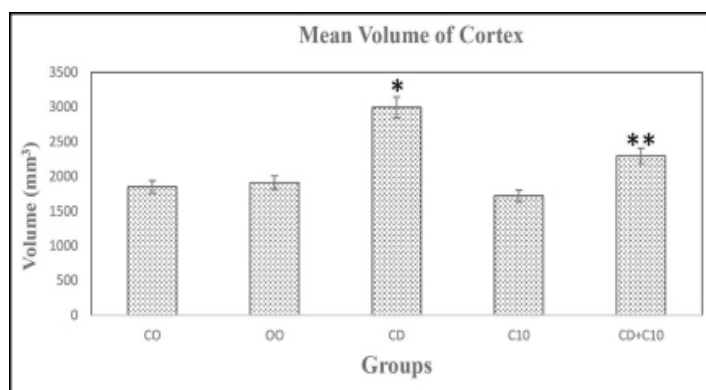


Figure 2. Graphs of the mean volumes of cortex in the CO, OO, CD, C10, and CD+C10 groups. *, a significant difference between the CD and CO groups; **, a significant difference between the CD+C10 and CD groups. CO, control; OO, olive oil; CD, cyclophosphamide; C10, coenzyme Q10; CD+C10, cyclophosphamide + coenzyme Q10

The mean medulla volumes of all groups are shown in Figure 3. Stereological results revealed that the mean volume of medulla was significantly higher in the CD group when compared with the CO group ($p < 0.01$). To the contrary, the mean volume of medulla in the CD+C10 group was significantly less when compared with the CD group ($p < 0.03$).

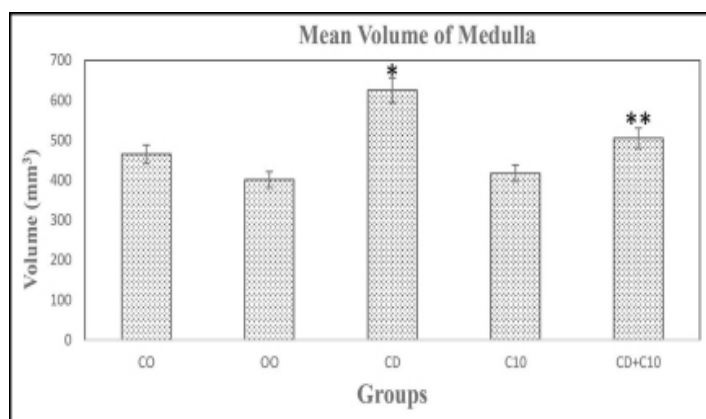


Figure 3. Graphs of the mean volumes of medulla in the CO, OO, CD, C10, and CD+C10 groups. *, a significant difference between the CD and CO groups; **, a significant difference between the CD+C10 and CD groups. CO, control; OO, olive oil; CD, cyclophosphamide; C10, coenzyme Q10; CD+C10, cyclophosphamide + coenzyme Q10

The mean glomerulus volumes of all groups are shown in Figure 4. The mean volume of glomeruli was significantly higher in the CD group when compared with the CO group ($p < 0.00$). We also observed a significant decrease in the CD+C10 group than the CD group ($p < 0.02$). Compared to the CO group, no difference was detected in the OO, C10, or CD+C10 groups.

The total volumes of proximal tubules of all groups are shown in Figure 5a. The total volume of proximal tubules was significantly reduced in the CD group when compared with the CO group (p

< 0.00). In the CD+C10 group, the total volume of distal tubules was significantly higher than the CD group ($p < 0.02$). Moreover, no difference was indicated in the OO, C10, and CD+C10 groups than the CO group.

The total volumes of distal tubules of all groups are shown in Figure 5b. We observed that the total volume of distal tubules was not significantly different in the CD group when compared with the CO group.

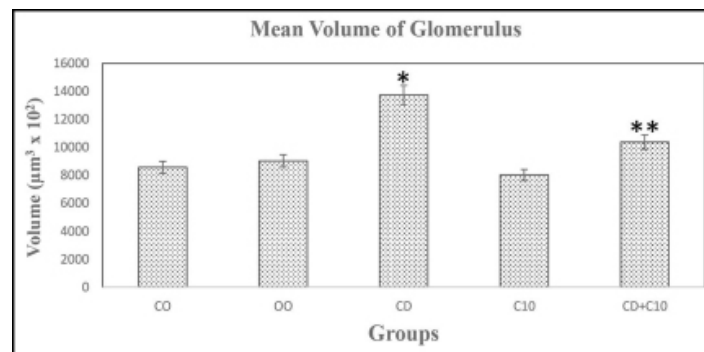


Figure 4. Graphs of the mean volumes of glomerulus in the CO, OO, CD, C10, and CD+C10 groups. *, a significant difference between the CD and CO groups; **, a significant difference between the CD+C10 and CD groups. CO, control; OO, olive oil; CD, cyclophosphamide; C10, coenzyme Q10; CD+C10, cyclophosphamide + coenzyme Q10

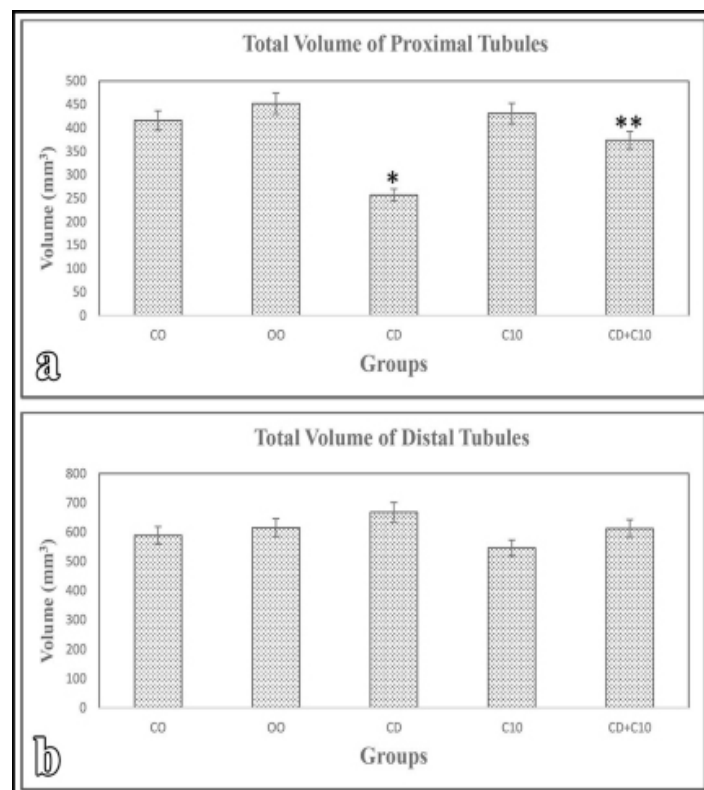


Figure 5. Graphs of the total volumes of proximal tubules (a) and distal tubules (b) in the CO, OO, CD, C10, and CD+C10 groups. *, a significant difference between the CD and CO groups; **, a significant difference between the CD+C10 and CD groups. CO, control; OO, olive oil; CD, cyclophosphamide; C10, coenzyme Q10; CD+C10, cyclophosphamide + coenzyme Q10

The volume fraction ratios of cortex to kidney of all groups are shown in Figure 6. We found that there was no significant difference in the volume fraction ratios of cortex to kidney between the CD, C10, or CD+C10 groups and the CO group.

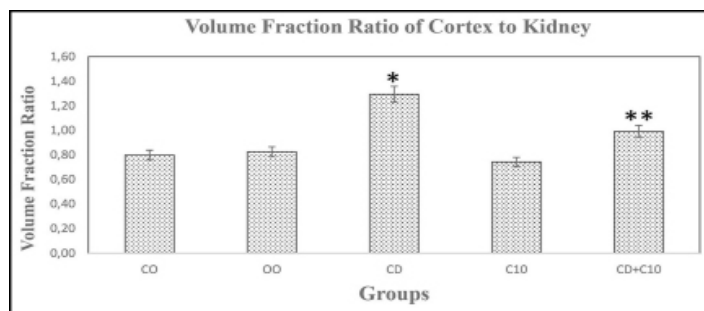


Figure 6. The volume fraction ratios of cortex to kidney in the CO, OO, CD, C10, and CD+C10 groups. CO, control; OO, olive oil; CD, cyclophosphamide; C10, coenzyme Q10; CD+C10, cyclophosphamide + coenzyme Q10

Histopathology

Histological structures of kidney tissues appeared healthy in the CO, OO, and C10 groups (Figure 7a, b and 8a, b). In the CD group, adverse effect of CD on kidney tissues was evident (Figure 7c-f). We found the mononuclear cell infiltration and dilated blood vessels with irregular wall in shape. Furthermore, congestion and enlargement of glomeruli were pronounced, and occasionally degeneration of tubular epithelial cells was detected. In the CD+C10 group, structural changes in kidney tissues were observed, but these were less than the CD group (Figure 8c, d).

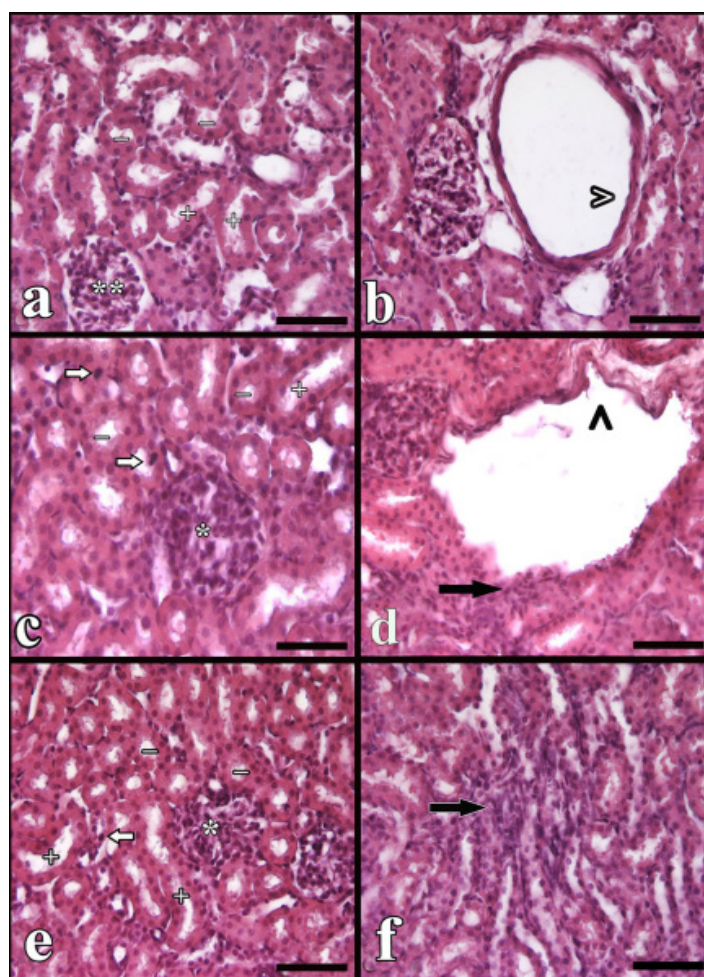


Figure 7. Light micrographs of kidney tissues in the CO (a), OO (b), and CD (c-f) groups. (+), normal distal tubules; (-), normal proximal tubules; (**), normal glomeruli; white arrowhead, normal shaped wall of blood vessel; black arrowhead, irregular shaped wall of blood vessel; (*), congestion and enlargement of glomeruli; black arrow, mononuclear cell infiltration; white arrow, degeneration of tubular epithelial cells. Groups: (CO), control; (OO), olive oil; (CD), cyclophosphamide. Scale bar = 50 μ m

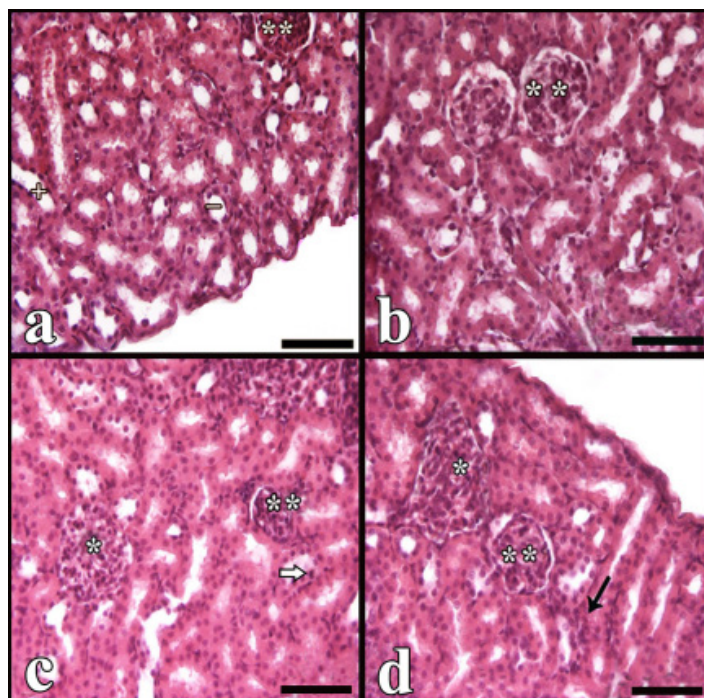


Figure 8. Light micrographs of kidney tissues in the C10 (a and b), and CD+C10 (c and d) groups. (+), normal distal tubules; (-), normal proximal tubules; (**), normal glomeruli; (*), congestion and enlargement of glomeruli; black arrow, mononuclear cell infiltration; white arrow, degeneration of tubular epithelial cell. Groups: (C10), coenzyme Q10; (CD+C10), cyclophosphamide + coenzyme Q10. Scale bar = 50 μ m

Discussion

Chemotherapy involves the administration of drugs that kill the cancer cells. After entering the bloodstream, these drugs pass through the body and kill all cancer cells. To overcome the resistance of human tumours to anticancer drugs, intensive chemotherapy is needed in tumour patients, especially with the use of high dose alkylating agents such as CD. However, the chemotherapeutic approach can induce toxic effects such as urotoxicity [17].

In the present study, we investigated the protective effect of C10 against the adverse effects of CD on the kidney tissues by means of the stereological technique. Unbiased stereological methods use accurate tool for estimating quantitative parameters. The Cavalieri methods provided reliable data regarding structure volume of interest in the kidney tissues.

Our stereological results revealed that the mean volumes of kidney, cortex, medulla, and glomeruli were significantly increased in the CD group than the CO group. Moreover, while the total volume of distal tubules was not different in the CD group than the CO group, there was a significant reduction in the total volume of proximal tubules. This reduced volume may have resulted from the toxic side effect induced by CD in the renal tissues. Our finding is consistent with earlier study that suggested a relationship between treatment with CD and increased renal toxicity [18]. Changes following a pathological event that occur in the organism are basically formed by the activation of specific mechanisms. Oxidative balance refers to the balance between formation and elimination of reactive oxygen species (ROS). Disruption of this balance results in oxidative stress that is responsible for unwanted complications in biosystem. It is thought that acrolein as an active metabolite of

CD causes oxidative toxicity via interfering with tissue antioxidant defence system [19]. Exposure to acrolein leads to the excessive formation of ROS that contributes to renal oxidative damage. ROS that includes superoxide anions, hydrogen peroxide, and hydroxyl radicals, can trigger harmful damage to macromolecules such as DNA, protein, and lipid membranes [20]. CD treatment can also cause a decrease in endogenous antioxidant activity and an increase in change in the renal tissues [21]. Katerji et al. [22] reported that CD therapy was associated with impairment of the endoplasmic reticulum function and damage to biological membrane. A recent study documented CD-induced oxidative DNA damage via activating apoptotic pathway, which elevated the expression of 8-hydroxy-2'-deoxyguanosine in the kidney tissues [23]. CD has also been thought to causes micronuclei formation and DNA strand breaks in renal tissue, resulting in genomic toxicity [24]. They also reported an inflammatory response after CD therapy via increasing inflammatory cytokines, and enzymes involved in ROS formation. Chloroacetaldehyde as a metabolite of CD can also contribute to nephrotoxicity through inducing oxidative stress [25].

We found in the CD+C10 group that administration of C10 significantly reduced the mean volumes of kidney, cortex, medulla, and glomeruli than the CD group. Furthermore, the total volume of proximal tubules was significantly increased in the CD+C10 group when compared with the CO group. These findings revealed the antioxidant potential of C10, which ameliorates the destructive change caused by CD in the kidney tissues. While studies on the antioxidant activity of C10 have mostly been performed on humans, there is limited research on animals. In addition to high oxygen consumption and metabolic activity, kidneys are susceptible to oxidant stress that exceeds the total antioxidant protection mechanism. C10, an antioxidant agent, can prevents or delay the effects of molecules that cause the oxidation of essential substances in the organism. Al-Megrin et al. [8] demonstrated that C10 supplementation ameliorated kidney injury caused by toxicants via reduction of inflammatory cytokines and apoptotic marker. Other study reported that mitochondria dysfunction in renal toxicity was improved by C10 treatment, which significantly increased the number of mitochondria and attenuated oxidative stress [26].

Histopathological results exhibited mononuclear cell infiltration, degeneration of tubular epithelial cells, and dilated blood vessels with irregular wall in shape, as well as congestion and enlargement of glomeruli in the CD group. This revealed that CD caused adverse effect on the kidney tissues, which was consistent with the previous studies. Ayhanci et al. [27] reported that CD caused histological damage to the kidney tissue via ROS formation. In the CD+C10 group, administration of C10 ameliorated the structural alterations caused by CD in the kidney tissues.

Our study limitation is that other parameters was not surveyed. Thus, we recommend that parameters supporting our volumetric results in the kidney tissues should be done in future projects.

Conclusion

Our findings showed that exposure to CD contributed to morphometrical and histological changes in the kidney tissues. Moreover, administration of C10 ameliorated such destructive alterations caused by CD therapy in kidneys. We suggest that the

usage of antioxidant agents may be beneficial to prevent toxic effects of CD.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Ethical approval of this study was granted by Laboratory Animal Ethics Committee of Gazi University)

References

1. Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. *Cancer Drug Resist.* 2019;2:141-60.
2. Ramadan G, El-Beih NM, Zahra MM. Egyptian sweet marjoram leaves protect against genotoxicity, immunosuppression and other complications induced by cyclophosphamide in albino rats. *Br J Nutr.* 2012;108:1059-68.
3. Iqbal A, Iqbal MK, Sharma S, et al. Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: Old drug with a new vision. *Life Sci.* 2019;218:112-31.
4. Ponticelli C, Escoli R, Moroni G. Does cyclophosphamide still play a role in glomerular diseases? *Autoimmun Rev.* 2018;17:1022-7.
5. Shruthi S, Shenoy KB. Gallic acid: A promising genoprotective and hepatoprotective bioactive compound against cyclophosphamide induced toxicity in mice. *Environ Toxicol.* 2020 Sep 9.
6. Ayza MA, Zewdie KA, Tesfaye BA, et al. The Role of antioxidants in ameliorating cyclophosphamide-induced cardiotoxicity. *Oxid Med Cell Longev.* 2020;2020:4965171.
7. Al-Megrin WA, Soliman D, Kassab RB, et al. Coenzyme Q10 Activates the antioxidant machinery and inhibits the inflammatory and apoptotic cascades against lead acetate-induced renal injury in rats. *Front Physiol.* 2020;11:64.
8. Saito Y, Kumamoto T, Shiraiwa M, et al. Cyclophosphamide-induced hemorrhagic cystitis in young patients with solid tumors: A single institution study. *Asia Pac J Clin Oncol.* 2018;14:e460-4.
9. Rodick TC, Seibels DR, Babu JR, et al. Potential role of coenzyme Q10 in health and disease conditions. *Nutrition and Dietary Supplements* 2018;2018:1-11.
10. Sangsefidi ZS, Yaghoubi F, Hajiahmadi S, et al. The effect of coenzyme Q10 supplementation on oxidative stress: A systematic review and meta-analysis of randomized controlled clinical trials. *Food Sci Nutr.* 2020;8:1766-76.
11. Abraham P, Isaac B. The effects of oral glutamine on cyclophosphamide-induced nephrotoxicity in rats. *Hum Exp Toxicol.* 2011;30:616-23.
12. İlhan A, Yolcu U, Oztas E, et al. Therapeutic effects of proanthocyanidin and coenzyme Q10 on nitrogen mustard-induced ocular injury. *Arq Bras Oftalmol.* 2018;81:226-31.
13. Yahyazadeh A, Altunkaynak BZ. Effect of luteolin on biochemical, immunohistochemical, and morphometrical changes in rat spinal cord following exposure to a 900 mhz electromagnetic field. *Biomed Environ Sci.* 2020;33:593-602.
14. Yahyazadeh A, Altunkaynak BZ, Akgül N, et al. A histopathological and stereological study of liver damage in female rats caused by mercury vapor. *Biotech Histochem.* 2017;92:338-46.
15. Altunkaynak BZ, Akgul N, Yahyazadeh A, et al. A stereological study of the effects of mercury inhalation on the cerebellum. *Biotech Histochem.* 2019;94:42-7.
16. Yahyazadeh A, Altunkaynak BZ, Alkan I. The morphometrical and immunohistochemical investigation of the effect of topiramate on liver and the role of neuropeptide Y receptor in an obese female rat. *Bratisl Lek Listy.* 2020;121:656-62.
17. ALHaithloul HAS, Alotaibi MF, Bin-Jumah M, et al. Olea europaea leaf extract up-regulates Nrf2/ARE/HO-1 signaling and attenuates cyclophosphamide-induced oxidative stress, inflammation and apoptosis in rat kidney. *Biomed Pharmacother.* 2019;111:676-85.
18. Goudarzi M, Khodayar MJ, Hosseini Tabatabaei SMT, et al. Pretreatment

- with melatonin protects against cyclophosphamide-induced oxidative stress and renal damage in mice. *Fundam Clin Pharmacol*. 2017;31:625-35.
19. Moghe A, Ghare S, Lamoreau B, et al. Molecular mechanisms of acrolein toxicity: relevance to human disease. *Toxicol Sci*. 2015;143:242-55.
20. Yahyazadeh A, Deniz OG, Kaplan AA, et al. The genomic effects of cell phone exposure on the reproductive system. *Environmental research*. 2018;167:684-93.
21. El-Sebaey AM, Abdelhamid FM, Abdalla OA. Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. *Environ Sci Pollut Res Int*. 2019;26:15559-72.
22. Katerji M, Filippova M, Duerksen-Hughes P. Approaches and methods to measure oxidative stress in clinical samples: Research applications in the cancer field. *Oxid Med Cell Longev*. 2019;2019:1279250.
23. Caglayan C, Temel Y, Kandemir FM, et al. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. *Environ Sci Pollut Res Int*. 2018;25:20968-84.
24. Rehman MU, Tahir M, Ali F, et al. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of Ellagic acid. *Mol Cell Biochem*. 2012;365:119-27.
25. Chen L, Xiong X, Hou X, et al. Wuzhi capsule regulates chloroacetaldehyde pharmacokinetics behaviour and alleviates high-dose cyclophosphamide-induced nephrotoxicity and neurotoxicity in rats. *Basic Clin Pharmacol Toxicol*. 2019;125:142-51.
26. Yu JH, Lim SW, Luo K, et al. Coenzyme Q(10) alleviates tacrolimus-induced mitochondrial dysfunction in kidney. *FASEB J*. 2019;33:12288-98.
27. Ayhanci A, Günes S, Sahinturk V, et al. Seleno L-methionine acts on cyclophosphamide-induced kidney toxicity. *Biol Trace Elem Res*. 2010;136:171-9.