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Research Article
Cyclophosphamide-Induced Liver Damage and Effect of Coenzyme Q10: An Experimental Rat Study

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Abstract

Background and Objective: Although cyclophosphamide is a potent antineoplastic drug its' toxic effects such as damage to the liver, kidney and ovary diminished its widespread utilization. This trial was designed to assess the effect of coenzyme Q10 on cyclophosphamide-induced toxicity in rat liver. **Materials and Methods:** The rats were divided into 3 groups: In group 1 (control group) (n = 10), nothing was done, group 2 (cyclophosphamide group) (n = 10), 30 mg kg^{-1} intraperitoneal cyclophosphamide was administered for 7 days and group 3 (cyclophosphamide+coenzyme Q10 group) (n = 10), 30 mg kg^{-1} cyclophosphamide and 10 mg kg^{-1} coenzyme Q10 were given for 7 days via intraperitoneal route. Biochemical measurements (SOD and CAT activity and MDA level) were performed. The liver of the rats was surgically extirpated in all groups. Histopathological damage in the liver was evaluated. **Results:** The hepatocyte damage markers such as inflammation, hemorrhage, necrosis, edema, parenchymal damage and hepatocyte damage were more common in the cyclophosphamide group than cyclophosphamide+coenzyme Q10 group ($p<0.05$). The activities of SOD and CAT were lower and levels of MDA were higher in the cyclophosphamide group than in the cyclophosphamide+coenzyme Q10 group ($p<0.05$). **Conclusion:** Coenzyme Q10 was found to be efficient in preserving the liver tissue from cyclophosphamide-induced toxic effects.

Key words: Coenzyme Q10, cyclophosphamide, liver, rat, toxicity, Bcl-2, immunohistochemistry

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cyclophosphamide has been used for years in the treatment of many malignancies, especially solid tumors and lymphoma. However, the toxicity of the cyclophosphamide restricts its usage[1]. It turns into active metabolites such as acrolein and phosphoramide mustard in the hepatocytes[2]. Cruz-Valencia *et al.*[3], reported that acrolein was responsible for the hepatotoxicity due to cyclophosphamide. It increases reactive oxygen species (ROS) by enhancing the metabolism of lipids and inhibits the catalase (CAT) and superoxide dismutase (SOD) which were involved in the oxidation system[4]. It has been demonstrated that the increased ROS levels might lead to an imbalance of the oxidants-antioxidants system, an increase of the inflammatory cytokines, oxidative stress and apoptosis[5,6]. Therefore, it might be very useful to administer an antioxidant chemical to prevent cyclophosphamide-induced hepatotoxicity.

Coenzyme Q10 is an antioxidant substance playing an important role in biochemical reactions such as electron transport chain and redox reaction[7]. It has been shown to contain anti-inflammatory and anti-apoptotic activity[8]. Moreover, it has been claimed that coenzyme Q10 reduces ROS levels and prevents oxidative stress[9].

Hence, it was hypothesized that coenzyme Q10 might prevent the adverse effects due to cyclophosphamide. In this study, the efficacy of coenzyme Q10 on cyclophosphamide-induced hepatotoxicity was assessed.

MATERIALS AND METHODS

Study area: The study was carried out between April 2023 to June 2023 at Erciyes University Faculty of Medicine, Department of Histology and Embryology.

Ethical statement: The ethical report of the trial was taken from the Erciyes University Faculty of Medicine Local Ethics Committee. The number and date of the document were 23/028 and 01.02.2023.

Study design: Cyclophosphamide and coenzyme Q10 were obtained from a local pharmacy (Kirsehir, Turkey). The study was conducted at Erciyes University Faculty of Medicine, Department of Histology and Embryology. Rats were obtained from Erciyes University Experimental Research Application and Research Center Experimental Animal Unit. Thirty female Wistar-albino rats aged 10-12 weeks were allocated to the trial. The *ad libitum* method was utilized, with unrestricted access to food and water. All the rats were treated to a temperature between 20 and 22°C and a 12 hrs light/12 hrs dark cycle.

Experimental study: A total of 3 groups were created. No procedure was applied in group 1 (control group) (n = 10). Group 2 (cyclophosphamide group) (n = 10), 30 mg kg^{-1} intraperitoneal cyclophosphamide was administered for 7 days and nothing was done. Group 3 (cyclophosphamide+ coenzyme Q10 group) (n = 10), 30 mg kg^{-1} cyclophosphamide and 10 mg kg^{-1} coenzyme Q10 were administered for 7 days intraperitoneally.

Anesthesia procedure was performed by utilizing ketamine hydrochloride (45 mg kg^{-1}, Ketax, Vem İlaç, Ankara, Turkey) and xylazine hydrochloride (5 mg kg^{-1}, Rompun, Bayer, Berlin, Germany). Blood was collected from the heart tissue of all animals by direct puncture. The liver of the rats was surgically extirpated. A high dose of anesthetic was used to sacrifice the animals.

Biochemical measurement: The activities of SOD and CAT and levels of MDA were determined by utilizing a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The MDA levels were detected by using the thiobarbituric acid test[10]. The activities of SOD and CAT were measured in terms of the methods stated in prior studies[11,12].

Histopathological assessment: The liver tissues were fixed with 10% formaldehyde. Then paraffin embedding was performed. Sections with a thickness of 5 micrometers were stained with hematoxylin-eosin dye for microscopic analysis. Besides, immunohistochemical Bcl-2 staining was performed. Histopathological assessment was performed via a microscope (Olympus® Inc., Tokyo, Japan). The tissue damage was scored by determining the highest area. A modified semi-quantitative scoring was performed. Five groups were described (0: Absent, 1: Minimal, 2: Mild, 3: Moderate and 4: Severe). Hemorrhage, necrosis, edema, inflammation, parenchymal injury and hepatocyte damage were utilized to assess the liver damage.

Bcl-2 determination: The Bcl-2 dye was evaluated in four groups (0: no staining, 1: less than 10% nuclear staining in liver tissue, 2: 10-30% nuclear staining, 3: more than 30% nuclear staining).

Statistical analysis: Statistical analysis was performed with the help of Statistical Package for the Social Sciences (22.00 SPSS Inc., Chicago, Illinois). The sample size calculated using power analysis was determined with 80% accuracy and at least 8. Chi-square and independent t-tests were utilized for analyses. As $p<0.05$ was accepted as statistically significant.

RESULTS

The MDA levels in the cyclophosphamide group were statistically significantly higher than the cyclophosphamide+ coenzyme Q10 group ($p<0.05$). The SOD and CAT activities in the cyclophosphamide group were statistically significantly lower than the cyclophosphamide+coenzyme Q10 group ($p<0.05$) (Table 1).

The macroscopic appearance of the samples in all 3 groups was similar to each other. However, tissue damage markers were statistically significantly higher in the cyclophosphamide group than the cyclophosphamide+ coenzyme Q10 group ($p<0.05$) (Table 2). Scores showing histological damage such as inflammation, hemorrhage, necrosis, edema, parenchymal damage and hepatocyte damage were very prominent in the cyclophosphamide group. However, the addition of coenzyme Q10 resulted in an improvement in damage parameters ($p<0.05$). Morphology and structural features of liver tissue were found to be close to normal, especially in the cyclophosphamide+coenzyme Q10 group (Fig. 1).

Immunohistochemical examination using Bcl-2 dye showed that the parameters showing histological damage were higher in the cyclophosphamide group. As in the light microscope examination, the markers were found to be improved when coenzyme Q10 was given (Fig. 2).

Table 1: Blood levels of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in serum samples of the groups

	MDA (nmol mg^{-1})	SOD (U mg^{-1})	CAT (U mg^{-1})
Control group (n = 10)	10.72±3.11	21.4±7.61	44.05±11.23
Cyclophosphamide group (n = 10)	24.1±13.06*	7.22±1.54*	17.37±7.12*
Cyclophosphamide+coenzyme Q10 group (n = 10)	17.05±5.45*	13.16±4.35*	29.14±9.62*

*Significant difference ($p<0.05$) between groups 2 and 3, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase and data are presented as mean±SD

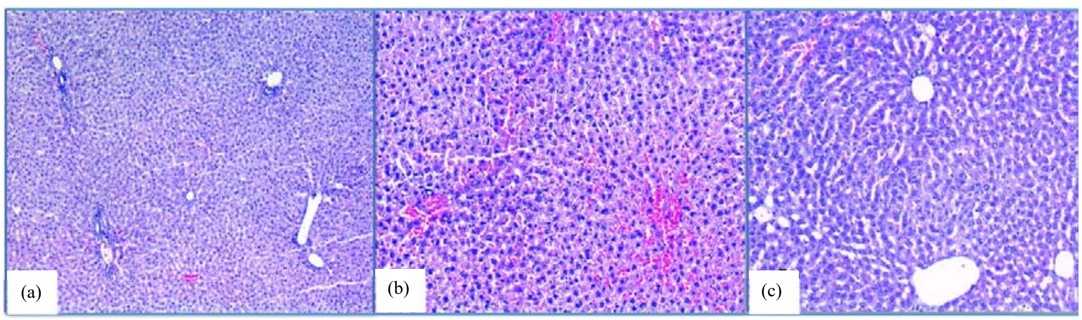

Fig. 1(a-c): Light microscopic appearance of liver tissue by using hematoxylin and eosin dye, (a) Control group, minimal hemorrhage in the ovarian stroma of the rat from the control group (H&E, ×50), (b) Cyclophosphamide group, the focal area of inflammation and hemorrhage within the liver parenchyma (H&E, ×50) and (c) Cyclophosphamide+ coenzyme Q10 group, mild congestion and inflammation in the parenchyma (H&E, ×50)

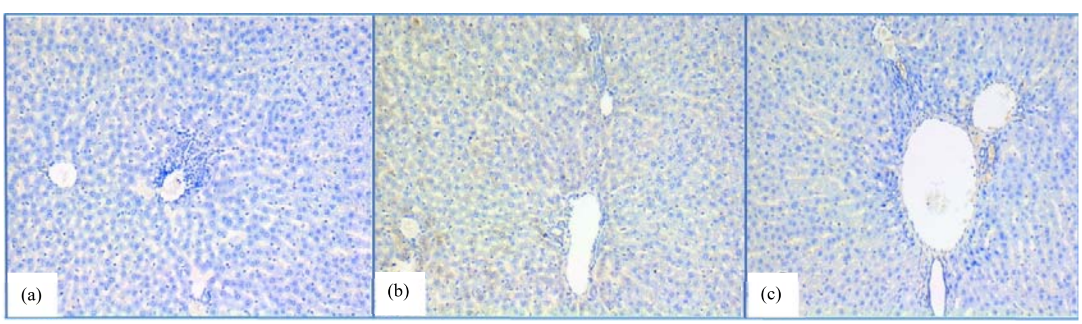

Fig. 2(a-c): Immunohistochemical staining with Bcl-2 dye, (a) Control group, mild edema in the liver tissue of the rat from the control group (×100), (b) Cyclophosphamide group, inflammation and edema within the liver parenchyma (×100) and (c) Cyclophosphamide+coenzyme Q10 group, a minimal to near-normal edema and inflammation within the liver parenchyma (×100)

Table 2: Distribution of histological damage according to the groups

	Control group (n = 10)	Cyclophosphamide group (n = 10)	Cyclophosphamide+coenzyme Q10 group (n = 10)
Hemorrhage	0.00	2.00*	1.00*
Necrosis	0.00	1.00	1.00
Edema	0.00	2.14*	1.08*
Inflammation	0.00	1.89*	1.11*
Parenchymal injury	0.00	2.45*	1.28*
Hepatocyte damage	0.00	2.25*	1.09*

*Significant difference ($p<0.05$) between groups 2 and 3, histopathological scoring was done by determining the highest area, four categories: 0: None, 1: Minimal, 2: Mild, 3: Moderate and 4: Severe were determined by making semi-quantitative analysis and the parameters were scored accordingly

DISCUSSION

This prospective randomized experimental study evaluated the effect of coenzyme Q10 on cyclophosphamide-induced damage in rat liver and found that coenzyme Q10 seems to reverse the toxicity due to cyclophosphamide. This is the first trial assessing the potential beneficial effect of coenzyme Q10 against liver toxicity in rats due to cyclophosphamide.

Shirani *et al.*[13] indicated that hepatic tissue is susceptible to toxic drugs such as cyclophosphamide due to its metabolism and detoxification processes. Emadi *et al.*[14] reported that cyclophosphamide usage is limited due to liver, kidney and bone marrow damage in long-term use. The main causative factor of impaired liver function is oxidative stress. Cyclophosphamide elevates the amount of harmful compounds and molecules. As a result of this series of reactions, the cellular antioxidant defense mechanism deteriorates[15,16]. The MDA levels increase and antioxidant enzymes such as SOD and CAT tend to be lower in the rats given cyclophosphamide due to the damage in the oxidative system. Kuang *et al.*[17] demonstrated that SOD and CAT are major protective enzymes and prevent liver injury due to oxidative stress. In this study, hepatocyte damage was more in the cyclophosphamide group than in other groups.

Coenzyme Q10 is a natural substance catalyzing many reactions such as the electron transport chain. It contains antioxidant properties and especially plays a role in reversing oxidative stress-related changes[18]. Cornelius *et al.*[8] reported that coenzyme Q10 inhibits the production of ROS and hydroxyl radicals, decreases apoptosis and consequently prevents oxidative damage. Furthermore, it has been shown that coenzyme Q10 interacts directly with alpha-tocopherol and neutralizes free radicals[19]. Because of all these potential benefits, we aimed to investigate the effect of coenzyme Q10 on cyclophosphamide-induced liver toxicity. The SOD and CAT reverse oxidative stress converting H_2O_2 into water and oxygen. Hence, the addition of an antioxidant such as coenzyme Q10 would be efficient to elevate the activities of SOD and CAT.

In this study, results showed that MDA levels tend to increase and the activities of SOD and CAT tend to decrease due to cyclophosphamide. The addition of coenzyme Q10 led to an elevation in the activities of SOD and CAT and a reduction in the levels of MDA. Histopathologic markers demonstrating damage were more prominent in the cyclophosphamide group. Coenzyme Q10 diminished the cyclophosphamide-induced tissue damage in rat liver. In immunohistochemical staining, the damage scores were prominently seen in the cyclophosphamide group and the addition of coenzyme Q10 l reversed this picture.

CONCLUSION

Coenzyme Q10 supplementation unquestionably reversed cyclophosphamide-induced liver damage. This was confirmed biochemically by measuring SOD, CAT and MDA levels. Likewise, the protective effect of coenzyme Q10 against cyclophosphamide toxicity was also demonstrated histopathologically. According to our short-term findings, coenzyme Q10 seems to improve liver damage due to cyclophosphamide.

SIGNIFICANCE STATEMENT

The purpose of this study is to prevent cyclophosphamide-induced hepatotoxicity by using coenzyme Q10. Coenzyme Q10 supplementation increased the activities of SOD and CAT and reduced the levels of MDA. Also, coenzyme Q10 diminished the cyclophosphamide-induced tissue damage in rat liver. All these findings suggest that coenzyme Q10 may be an option in the prevention and treatment of cyclophosphamide-induced liver damage.

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REFERENCES

1. Caglayan, C., Y. Temel, F.M. Kandemir, S. Yildirim and S. Kucukler, 2018. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. Environ. Sci. Pollut. Res., 25: 20968-20984.
2. Ghareeb, M.A., M. Sobeh, W.H. El-Maadawy, H.S. Mohammed, H. Khalil, S. Botros and M. Wink, 2019. Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato-renal protective potential against cyclophosphamide induced toxicity in mice. Antioxidants, Vol. 8. 10.3390/antiox8090415.
3. Cruz-Valencia, R., A.A. Arvizu-Flores, J.A. Rosas-Rodríguez and E.M. Valenzuela-Soto, 2021. Effect of the drug cyclophosphamide on the activity of porcine kidney betaine aldehyde dehydrogenase. Mol. Cell. Biochem., 476: 1467-1475.
4. Adikwu, E. and B. Bokolo, 2018. Effect of cimetidine on cyclophosphamide-induced liver toxicity in albino rats. Asian J. Med. Sci., 9: 50-56.
5. Lee, S.I. and K.S. Kang, 2017. Function of capric acid in cyclophosphamide-induced intestinal inflammation, oxidative stress, and barrier function in pigs. Sci. Rep., Vol. 7. 10.1038/s41598-017-16561-5.
6. Wang, X., M.A. Martinez, G. Cheng, Z. Liu and L. Huang *et al*., 2016. The critical role of oxidative stress in the toxicity and metabolism of quinoxaline 1,4-di-*N*-oxides *in vitro* and *in vivo*. Drug Metab. Rev., 48: 159-182.
7. Gendelman, M. and Z. Roth, 2012. Incorporation of coenzyme Q10 into bovine oocytes improves mitochondrial features and alleviates the effects of summer thermal stress on developmental competence. Biol. Reprod., Vol. 87. 10.1095/biolreprod.112.101881.
8. Cornelius, N., J.H. Wardman, I.P. Hargreaves, V. Neergheen and A.S. Bie *et al*., 2017. Evidence of oxidative stress and mitochondrial dysfunction in spinocerebellar ataxia type 2 (SCA2) patient fibroblasts: Effect of coenzyme Q10 supplementation on these parameters. Mitochondrion, 34: 103-114.
9. Yousef, A.O.S., A.A. Fahad, A.E. Abdel Moneim, D.M. Metwally, M.F. El-khadragy and R.B. Kassab, 2019. The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities. Int. J. Environ. Res. Public Health, Vol. 16. 10.3390/ijerph16162895.
10. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
11. Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.
12. Aebi, H., 1984. Catalase *in vitro*. In: Methods in Enzymology, Packer, L., Academic Press, Cambridge, Massachusetts, United States, ISBN: 9780121820053, pp: 121-126.
13. Shirani, K., B.S. Yousefsani, M. Shirani and G. Karimi, 2020. Protective effects of naringin against drugs and chemical toxins induced hepatotoxicity: A review. Phytother. Res., 34: 1734-1744.
14. Emadi, A., R.J. Jones and R.A. Brodsky, 2009. Cyclophosphamide and cancer: Golden anniversary. Nat. Rev. Clin. Oncol., 6: 638-647.
15. Li, B., W. Li, Y. Tian, S. Guo, L. Qian, D. Xu and N. Cao, 2020. Selenium-alleviated hepatocyte necrosis and DNA damage in cyclophosphamide-treated geese by mitigating oxidative stress. Biol. Trace Elem. Res., 193: 508-516.
16. Khairnar, S.I., U.B. Mahajan, K.R. Patil, H.M. Patel and S.D. Shinde *et al*., 2020. Disulfiram and its copper chelate attenuate cisplatin-induced acute nephrotoxicity in rats via reduction of oxidative stress and inflammation. Biol. Trace Elem. Res., 193: 174-184.
17. Kuang, H., Y. Jiao, W. Wang, F. Wang and Q. Chen, 2020. Characterization and antioxidant activities of intracellular polysaccharides from *Agaricus bitorquis* (QuéL.) Sacc. Chaidam ZJU-CDMA-12. Int. J. Biol. Macromol., 156: 1112-1125.
18. Aaseth, J., J. Alexander and U. Alehagen, 2021. Coenzyme Q_{10} supplementation-in ageing and disease. Mech. Ageing Dev., Vol. 197. 10.1016/j.mad.2021.111521.
19. Manzar, H., D. Abdulhussein, T.E. Yap and M.F. Cordeiro, 2020. Cellular consequences of coenzyme Q10 deficiency in neurodegeneration of the retina and brain. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21239299.