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Assessing perfluorooctanoic acid toxicity in lung, heart, and testis tissues of mice: Evaluation of protective effects of taurine and coenzyme Q_{10}

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ABSTRACT: Perfluorooctanoic acid (PFOA) is a persistent compound which is associated with many negative effects on human health. In this study, possible oxidative stress inducing effects of PFOA and protective effects of taurine and coenzyme Q_{10} (CoQ_{10}) in lung, heart and testes of mice were investigated. Animals were administered 15 and 30 mg/kg doses of PFOA orally for 10 days. All three organ weights were found to be affected in response to PFOA while taurine seemed to be effective against absolute heart weight reduction. PFOA was also found to induce histopathological changes in lung tissue. 15 mg/kg of PFOA reduced catalase (CAT) activity in lung; increased malondialdehyde (MDA) and total glutathione (GSH) levels, activities of superoxide dismutase (Cu-Zn SOD) and CAT in heart; and increased GSH levels and reduced Cu-Zn SOD and CAT activities in testes. On the other hand, 30 mg/kg PFOA treatment led to Cu-Zn SOD activity increase in lung; GSH level and Cu-Zn SOD activity increase in heart; and reduction of all three enzyme activities in testes. Taurine was found to be protective against Cu-Zn SOD activity increase in both lung and heart tissues, as well as against reduction of glutathione peroxidase (GPx) and CAT activities in the testes. CoQ_{10} seemed to protect against PFOA-induced increases in MDA levels in the heart tissue. These results suggest the oxidative stress involvement in the toxicity mechanism of an important environmental pollutant, PFOA, in lung, heart and testes of mice. Moreover, taurine and CoQ_{10} have protective role to some extent against PFOA-induced toxicity.

KEYWORDS: Perfluorooctanoic acid; oxidative stress; pulmonary toxicity; cardiotoxicity; reproductive toxicity; taurine; coenzyme Q_{10}

1. INTRODUCTION

Perfluorinated substances are a large group of synthetic chemicals with wide range of applications and uses in the industry as well as household products. Strong carbon-fluorine bonds make these compounds non-biodegradable, thus causing their bioaccumulation and environmental persistence [1-3]. Perfluorooctanoic acid (PFOA), a member of this group, has been used since 1940s [3]. Aside from occupational exposure, main exposure sources to this chemical are contaminated water, foods, use of PFOA-containing products and dust inhalation [1, 2, 4]. Human exposure is estimated as 0.3–150 ng/kg daily [5].

PFOA has a long half-life (3.8 years) in humans, and it is eliminated without being metabolized [6]. Geometric mean of human serum PFOA concentrations were measured as 1.42 ng/ml in the United States [7] whereas serum level of 114,100 ng/ml was reported in an occupationally exposed worker [8]. Studies showed that PFOA was distributed mainly to liver, plasma, and kidney in animals [9, 10]. In a recent study made on mice, PFOA levels were found highest in the lung, after liver and blood [11].

The widespread presence of PFOA in the environment has led to it being monitored for various potential health effects. The International Agency for Research on Cancer (IARC) classified PFOA as possibly carcinogenic to humans (Group 2B) (2016) [12]. Epidemiological studies suggest that there is a positive association between PFOA exposure and many diseases, including carcinogenesis, thyroid and chronic kidney disease, hepatotoxicity, increased risk of obesity, and adverse effects on the immune system [5].

A growing body of evidence from animal studies indicates hepatotoxic effects of PFOA [13, 14]. However, the number of studies investigating the effects of PFOA on other tissues is relatively small. The

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lung is one of the target organs for PFOA. In addition, it is known that inhalation is one of the main routes of exposure to this substance, especially in the workplace and in people at home [5, 15]. Studies on the toxicity of PFOA in the lungs have different and contradictory results. Some studies found that PFOA exposure was associated with impaired lung function [16], while other studies found no association or insufficient evidence between PFOA exposure and lung disease in humans [17, 18]. Some animal studies in the literature suggest that prenatal PFOA exposure impairs lung development [19] or exacerbates inflammation in asthmatic mice [20]. In humans, PFOA exposure has been associated with high cholesterol [21] and cardiovascular disease [22], and in animals, a limited number of studies demonstrated developmental cardiotoxic effects of PFOA [23, 24]. With respect to the reproductive system, PFOA is classified as an endocrine disrupting chemical (EDC), and EDCs are known to have adverse effects on the male reproductive system [25, 26]. PFOA can affect sperm quality, reproductive hormone levels, and spermatogenesis in humans [27, 28]. Several *in vivo* studies have shown that PFOA exposure can lead to adverse effects on the testes and the male reproductive system [26, 29, 30].

Oxidative stress is one of the proposed toxicity mechanisms for PFOA. Several studies reported an association between PFOA exposure and oxidative stress [13, 30, 31]. To our knowledge, there are no comprehensive studies in rodents on the oxidative stress-inducing effects of PFOA in lung and heart tissues. In the present study, we investigated the oxidative stress-inducing effects of PFOA on lung, heart, and testicular tissues of Balb/c mice in addition to histopathological analysis of these tissues. The effects of taurine and coenzyme Q_{10} (CoQ_{10}) on PFOA-induced toxicity were also studied in the same systems.

2. RESULTS

2.1. Organ weights

Relative lung weight of animals significantly increased (13%, $p< 0.05$) in response to 30 mg/kg PFOA treatment, although no significant change was observed in absolute lung weight. Absolute heart weight significantly and dose-dependently decreased in both PFOA dose treatment groups (1.2 and 1.5-fold, $p< 0.05$ and $p< 0.001$, respectively), whereas relative heart weight was significantly reduced only by the 30 mg/kg PFOA dose (21%, $p< 0.05$). On the other hand, absolute testes weight was significantly reduced only by 15 mg/kg PFOA treatment (1.29-fold, $p< 0.05$). Relative testes weight was not affected significantly in neither PFOA dose group. Taurine seemed to protect against absolute heart weight reduction, while CoQ_{10} was not found effective against any weight changing effect of PFOA in lung and heart (Table 1).

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Table 1. Effects of oral PFOA, CoQ_{10} and taurine administration on relative lung, heart and testes weights of mice.

Groups	Lung Weight (g)	Relative Lung Weight (%)	Heart Weight (g)	Relative Heart Weight (%)	Testes Weight (g)	Relative Testes Weight (%)
Control (water)	0.20 ± 0.01	0.61 ± 0.01	0.18 ± 0.01	0.56 ± 0.05	0.22 ± 0.01	0.67 ± 0.06
PFOA (15 mg/kg)	0.19 ± 0.01	0.66 ± 0.05	0.15 ± 0.01*	0.53 ± 0.03	0.17 ± 0.01*	0.58 ± 0.01
PFOA (30 mg/kg)	0.18 ± 0.01	0.69 ± 0.03*	0.12 ± 0.01**	0.44 ± 0.02*	0.21 ± 0.01	0.81 ± 0.03
Taurine	0.21 ± 0.01	0.66 ± 0.04	0.18 ± 0.01	0.57 ± 0.03	0.19 ± 0.01	0.60 ± 0.02
Taurine + PFOA	0.20 ± 0.01	0.69 ± 0.03	0.14 ± 0.01#	0.49 ± 0.03	0.19 ± 0.02#	0.64 ± 0.03##
Control (corn oil)	0.21 ± 0.01	0.69 ± 0.04	0.17 ± 0.01	0.54 ± 0.02	0.21 ± 0.01	0.69 ± 0.04
CoQ_{10}	0.22 ± 0.02	0.67 ± 0.06	0.15 ± 0.01	0.46 ± 0.04+	0.24 ± 0.01	0.71 ± 0.04
CoQ_{10} + PFOA	0.17 ± 0.01	0.64 ± 0.06	0.13 ± 0.01	0.47 ± 0.03	0.21 ± 0.02	0.75 ± 0.06

Data expressed as mean ± SEM. *p < 0.05, **p < 0.01 relative to control (water) group; #p< 0.05, ##p< 0.01 relative to PFOA (30 mg/kg) group; +p< 0.05 relative to control (corn oil) group. n=6 for two control groups, n=7 for other groups.

2.2. Histopathological changes

In the lung, pseudostratified columnar epithelium lined bronchioles were at normal thickness in all groups (Figure 1). In all experimental groups, there were hemorrhage and congestion. This might be partially explained by asphyxia at the moment of death. In all single PFOA-treated mice groups, alveolar hemorrhage and more severe congestion were observed, in addition to pulmonary collapse which was seen at 30 mg/kg single and taurine pretreated PFOA group (Figure 1B, C, E). In taurine and CoQ_{10} pretreated groups, hemorrhage and congestion were also seen. However, at CoQ_{10} pretreated group, pulmonary collapse was observed to be less severe relative to single PFOA-treated group (Figure 1H). The histological examination of the heart tissue in the whole groups was found to be in the normal morphological appearance which exhibited myocardial fibers with centrally localized nuclei (Figure 2). In the testis tissue, mice in all experimental groups demonstrated normally arranged spermatogenic cells at various developmental stages inside seminiferous tubules (Figure 3).

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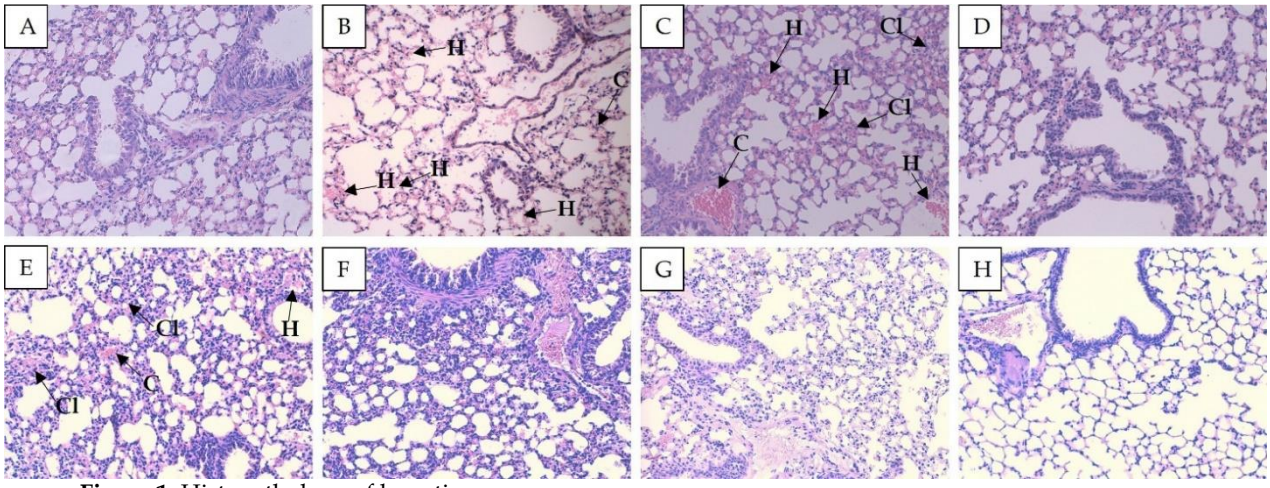

Figure 1. Histopathology of lung tissue.
(A) control (water); **(B)** 15 mg/kg PFOA; **(C)** 30 mg/kg PFOA; **(D)** taurine; **(E)** taurine+30 mg/kg PFOA; **(F)** control (corn oil); **(G)** CoQ_{10}; **(H)** CoQ_{10}+30 mg/kg PFOA. Arrows: C- congestion; H- hemorrhage; Cl- collapse (H&E staining, ×200 magnification).

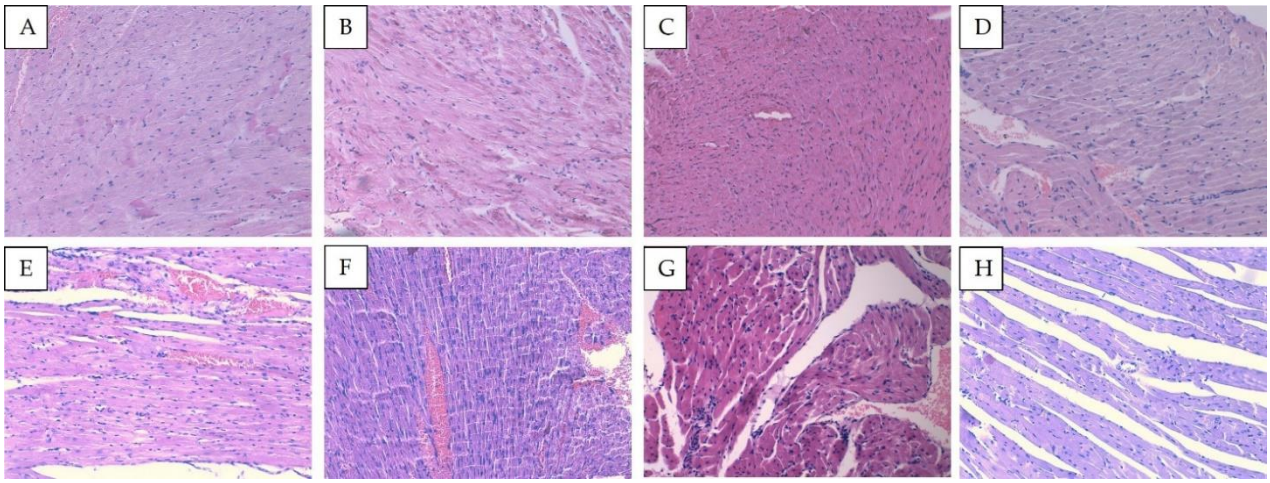

Figure 2. Histopathology of heart tissue.
Normal appearance of myocardial fibers with centrally localized nuclei was observed in all groups. **(A)** control (water); **(B)** 15 mg/kg PFOA; **(C)** 30 mg/kg PFOA; **(D)** taurine; **(E)** taurine+30 mg/kg PFOA; **(F)** control (corn oil); **(G)** CoQ_{10}; **(H)** CoQ_{10}+30 mg/kg PFOA (H&E staining, ×200 magnification).

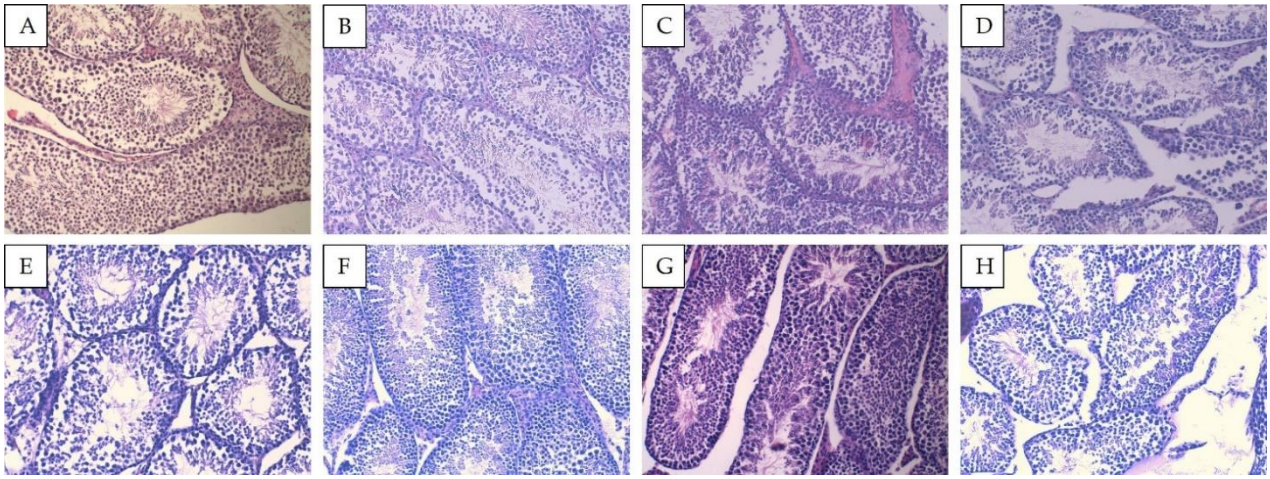

Figure 3. Histopathology of testicular tissue.
Regularly arranged seminiferous tubules were observed in all groups. **(A)** control (water); **(B)** 15 mg/kg PFOA; **(C)** 30 mg/kg PFOA; **(D)** taurine; **(E)** taurine+30 mg/kg PFOA; **(F)** control (corn oil); **(G)** CoQ_{10}; **(H)** CoQ_{10}+30 mg/kg PFOA (H&E staining, ×200 magnification).

2.3. Oxidative stress markers and antioxidant enzyme activities

In lung tissue, malondialdehyde (MDA) and total glutathione (GSH) levels were not impacted in both PFOA groups. Copper-zinc superoxide dismutase (Cu-Zn SOD) activity significantly rose by 22% following 30 mg/kg PFOA treatment, while catalase (CAT) activity decreased by 30% in response to 15 mg/kg PFOA administration ($p< 0.05$). Glutathione peroxidase (GPx) activity did not change in response to PFOA. When antioxidant effects were analyzed, only taurine was found to have protection in the lung tissue against SOD activity increase resulting from PFOA treatment (Figure 4).

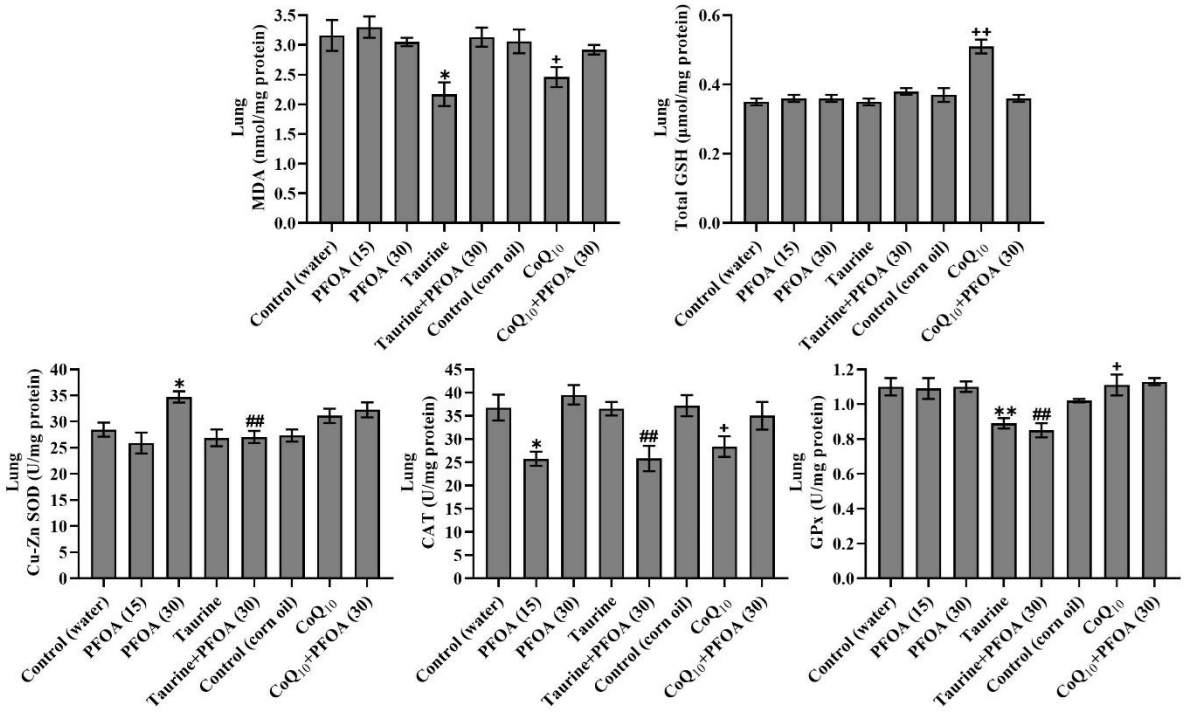

Figure 4. Oxidative stress related parameters levels in mice lung tissues treated with ig, PFOA, taurine and CoQ_{10}.
Data showed as mean ± SEM. *$p< 0.05$, **$p< 0.01$ relative to control (water) group; ##$p< 0.01$ relative to 30 mg/kg PFOA group; +$p< 0.05$, ++$p< 0.01$ relative to control (corn oil) group. n=6 for two control groups, n=7 for other groups.

MDA levels were significantly and 5% higher at only lower dose PFOA group ($p< 0.05$) in the heart tissue. Total GSH levels, on the other hand, were measured 18% higher relative to control in both PFOA treated groups ($p< 0.01$ and $p< 0.05$, respectively). Activities of SOD and CAT increased significantly after 15 mg/kg PFOA (8% and 18%, respectively; $p< 0.05$). Accordingly, 30 mg/kg PFOA treatment led to significant and 22% increase in SOD and 14%, albeit insignificant, increase in CAT activity. In contrast, GPx activity was not affected by PFOA in the same tissue. CoQ_{10} was found to be protective against MDA increasing effect of PFOA in the heart. Similar to its effect on lung, taurine reversed the SOD activity increasing effect of PFOA in the heart (Figure 5).

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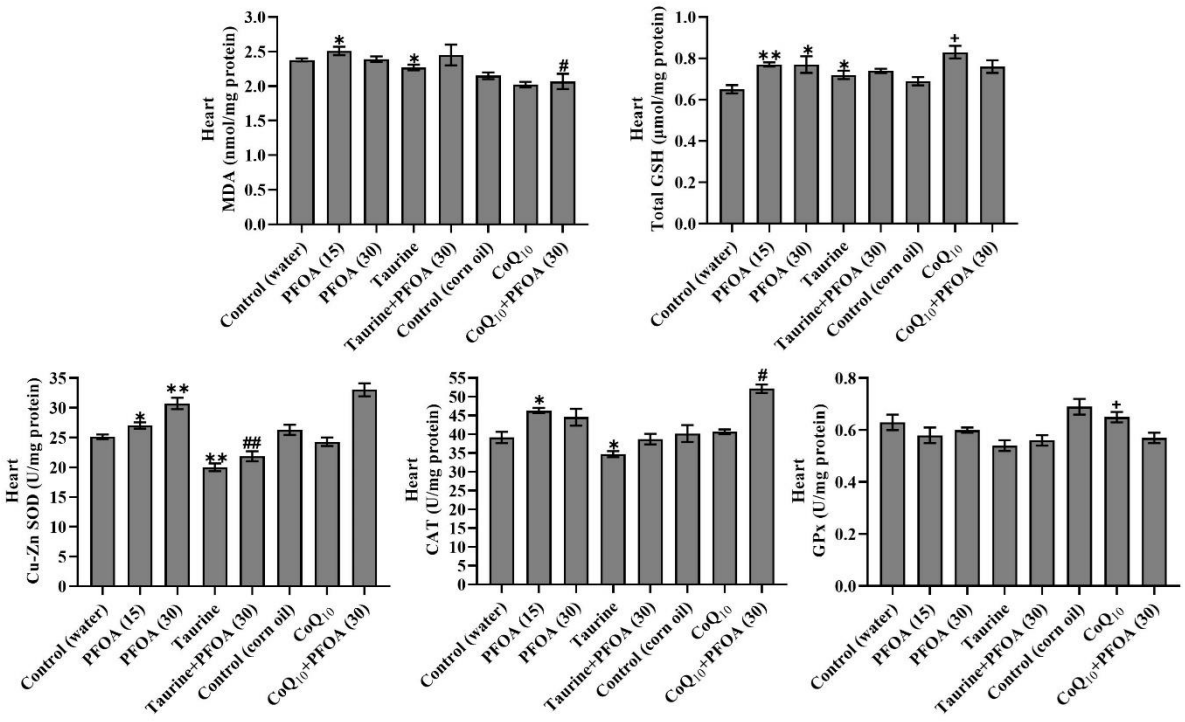

Figure 5. Oxidative stress related parameters levels in mice heart tissues given PFOA, taurine and CoQ_{10}. Data showed as mean ± SEM. *p< 0.05, **p< 0.01 relative to control (water) group; #p< 0.05, ##p< 0.01 relative to 30 mg/kg PFOA group; +p < 0.05 relative to control (corn oil) group. n=6 for two control groups, n=7 for other groups.

In testes, MDA levels were not altered by either dose of PFOA treatment, while total GSH levels were measured 27% higher relative to control at 15 mg/kg PFOA group (p< 0.01). As seen in Figure 6, all three enzyme activities were reduced in a dose-dependent manner after PFOA treatment. Dose-dependence was more pronounced in CAT activity. Rates of decrease were 12% and 13%; 13% and 25%; 9% and 12% for Cu-Zn SOD, CAT and GPx, respectively, with p < 0.05, except for CAT activity at 15 mg/kg PFOA group, which is non-significant. Taurine had a significant (p< 0.05) protective impact on GPx and CAT activities in testes (Figure 6).

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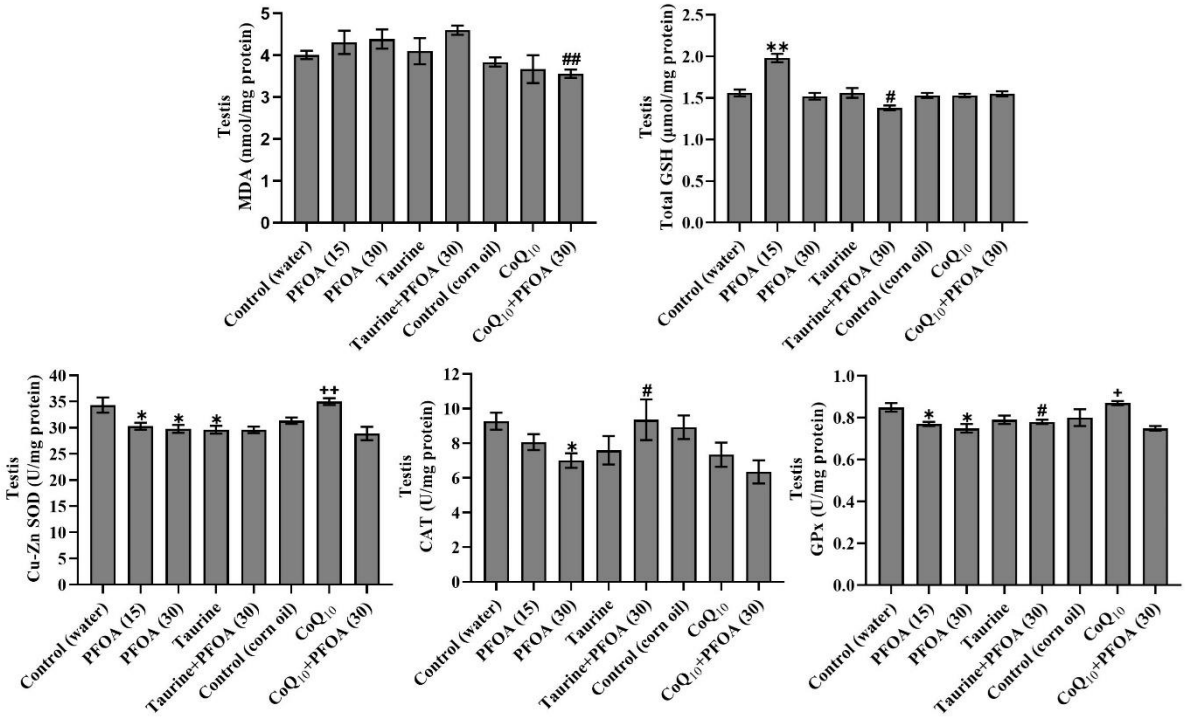

Figure 6. Oxidative stress related parameters levels in mice testis tissues given PFOA, taurine and CoQ_{10}. Data showed as mean ± SEM. *p< 0.05, **p< 0.01 relative to control (water) group; #p< 0.05, ##p< 0.01 relative to 30 mg/kg PFOA group; +p< 0.05, ++p< 0.01 relative to control (corn oil) group. n=6 for two control groups, n=7 for other groups.

3. DISCUSSION

In today's world, PFOA is an important health concern for humans and environment. [1, 5, 32]. Majority of previous studies focused on hepatotoxic effects of this chemical [33]. However, effects of PFOA on other organs and tissues, including lung, heart, and testes, may be critical given distribution of the substance and results of epidemiological studies of adverse health effects in these organs [11, 16, 22, 28].

There are limited number of studies with PFOA comparing weight and histopathological changes of lung and heart tissues of animals. In one of these studies, Rosen et al. [34] showed that 10 mg/kg PFOA did not cause any change in histological appearance of fetal mouse lung. In another study with rats, 5 and 20 mg/kg PFOA was given to animals for 28 days and similar to our observations, pulmonary congestion was seen, especially in the higher dose group in the lungs [35]. In the same study, differently from our data, focal or diffuse thickened epithelial walls, cellular infiltration and vasodilatation were also shown. It could be suggested that the difference between this and our study might be related to longer administration time of PFOA. In the current investigation, CoQ_{10} had some protection against pulmonary collapse induced by PFOA. Similarly, improvement of histological picture by CoQ_{10} against another substance, methotrexate-induced injury in lung tissue of rats was shown by Mohamed et al. [36].

As for the heart tissue, we did not detect histopathological changes because we examined one heart section and not the entire ventricular thickness. In the literature, Jiang et al [23] found that PFOA exposure resulted in thinning of the right ventricle in chicken embryos and hatchlings, but in contrast to our results, no change in relative heart weight was observed. In another study, it was shown that ventricular wall thickness was significantly reduced in chicken hatchlings and L-carnitine co-exposure preserved the hearts from these changes [37]. Although ventricular thickness was not measured in the present study, the dose-dependent decrease in absolute and relative heart weight may be due to ventricular wall thinning. A protective effect of taurine against the PFOA-induced reduction in heart weight was also demonstrated in the current study.

In agreement with other studies [38, 39], we found that absolute testes weights of animals were reduced following PFOA treatment while there was no significant effect on relative testis weight. As Yuan et al. [30] discussed in their study, potential reason of decrease in testicular weight may be the reduction in testosterone levels in response to PFOA exposure. They [30] also showed that exposure of 10 mg/kg PFOA

for 21 days to mice caused obvious histopathological changes in testes of mice and quercetin treatment attenuated these pathological changes. The short exposure time chosen in our study may be one of a reason why we did not observe histopathological changes in the testicles of mice with any treatment.

Oxidative stress is considered as one of the most important causes of many diseases including respiratory, cardiovascular, and reproductive systems. In the present study, lipid peroxidation was measured in terms of MDA, an indicator of oxidative stress. In addition, as a key physiologic antioxidant, total GSH levels [40] as well as activities of important antioxidant enzymes SOD, CAT and GPx which participate in first line defense of body against oxidative stress [41] were also determined. Here, we found that PFOA (30 mg/kg) increased SOD activity in the lungs whereas at 15 mg/kg reduced activity of CAT. Levels of MDA and GSH as well as activity of GPx were not observed to be affected by PFOA treatment. This finding seems plausible since GPx detoxifies hydrogen peroxides and lipid peroxides using GSH. Since SOD and CAT represent the first line of antioxidant protection, the changes in their activities could be explained by their preventive effect against the formation of lipid peroxidation by scavenging reactive oxygen species (ROS). The increase in SOD activity in the lung and the protective effect of taurine against this increase may indicate an initial defense of cells against PFOA-induced oxidative damage. In a study we could find in the literature that investigated the relationship between PFOA exposure and oxidative stress in lung cells, it was also shown that PFOA-induced ROS formation was eliminated by N-acetylcysteine [15].

In cardiac tissue, we showed that MDA and GSH levels, as well as all enzyme activities except GPx, increased in response to PFOA treatment. CoQ_{10} attenuated the MDA-increasing effect of PFOA, while taurine, like lung tissue, protected against the increase in SOD activity in the heart. In its function as an electron transmitter in the mitochondrial respiratory chain, CoQ_{10} acts as an antioxidant by scavenging free radicals, preventing the formation and spread of lipid peroxidation in cell membranes, and supporting the regeneration of α-tocopherol [42, 43]. It is known that mitochondria account for approximately 30% of the volume of mammalian cardiac muscle and are involved in the regulation of ROS in addition to energy production and other important effects [44-46]. Considering some characteristics of cardiac tissue mentioned above, the protective effect of CoQ_{10} against the lipid peroxidation-inducing effects of PFOA in cardiac tissue becomes more important. Moreover, the mitochondrion has been found to be a target organelle for PFOA [47]. Therefore, besides to CoQ_{10}, protective effects of taurine with its ability to improve mitochondrial function might also be considerable. In the literature, two of few studies investigating PFOA exposure on heart tissue found that PFOA led to ROS formation at chicken embryo cardiomyocytes and mitochondria isolated from mouse fetus heart [23, 24]. Additionally, similar to our results with antioxidants, in an *in vitro* study, L-carnitine was found to have protective effects against PFOA-induced ROS production in chicken embryo hearts as well as hatchling chicken hearts [48].

The testes are particularly vulnerable to oxidative stress due to the abundance of polyunsaturated fatty acids and the existence of potential ROS-producing systems [49, 50]. Increased GSH levels and decreased activities of all antioxidant enzymes in the testes of the mice measured in the current study suggest that oxidative stress in the testes may be an important cause of PFOA toxicity in this tissue. In the present study, the observed increase in GSH content in testicular tissues at low dose (15 mg/kg) may be due to the initial defense mechanism of the cells, whereas the decrease in GSH content at higher dose suggests depletion of GSH content in response to further increasing oxidative stress. In addition, the dose-dependent decrease in enzyme activities after exposure to PFOA suggests that oxidative stress overwhelms the antioxidant capacity of all these antioxidant enzymes. Although H_2O_2 levels were not measured in the present study, alleviation of GPx and CAT activities by taurine, a H_2O_2 scavenger, might be considered as supportive evidence for the involvement of H_2O_2 in oxidative stress inducing effect of PFOA in testis. In accordance with our results, PFOA treatment (10 mg/kg) for 21 days decreased activities of SOD and GPx in testis of mice, and these alterations were improved by simultaneous quercetin treatment [30]. In another study, it has been shown that PFOA treatment (2.5, 5, or 10 mg/kg) caused increases in MDA levels, and inhibition in activities of SOD and CAT in mice testis tissues [38].

In the present study, it was observed that both antioxidants provided some protection against PFOA-induced toxicity. Taurine proved to be protective for some oxidative stress parameters in all three tissues. CoQ_{10}, on the other hand, showed its protective effect mainly in cardiac tissues, probably due to the characteristic properties of cardiac tissues, as explained above. Actually, we expected that taurine and CoQ_{10} would have stronger antioxidant effects on other oxidative stress parameters as well. However, our model system, in which taurine and CoQ_{10} were pretreated rather than treated together, might be a reason for the observed lower protective effect. This aspect will be considered in our future studies.

4. CONCLUSION

In summary, we have shown that PFOA affects the balance between oxidants and antioxidants in all three tissues of mice to different degrees. The protection obtained with taurine and CoQ_{10} in some parameters could be considered as a confirmation of the oxidative stress inducing effect of this chemical. Considering the accumulation potential of PFOA in the lungs and its adverse effects on the cardiovascular and reproductive systems, it is clear that further studies are needed to clarify the mechanism of toxicity of this environmental pollutant. We believe that the results of the present study will contribute to the understanding of the mechanisms of the adverse effects of PFOA.

5. MATERIALS AND METHODS

5.1. Chemicals

All chemicals including PFOA (ammonium salt of perfluorooctanoic acid, 96%), taurine and CoQ_{10} were obtained from Sigma-Aldrich/Merck KGaA (Darmstadt, Germany).

5.2. Animals and experimental design

In this study, 30-35 g weighted male Balb/c mice, which are 10-week-old, were used. Mice were provided by Erciyes University Animal Care Unit. Animals were given free access to standard laboratory chow and tap water. 20 - 24°C temperature, 40 - 50% relative humidity and 12h-light/dark cycle was provided for animal housing conditions. Erciyes University Animal Ethics Committee approved the protocol used in this study (Decision Number: 13/33). All animal procedures agree with directive 2010/63/EU.

Eight animal groups were determined for this study. Six mice were found in 2 control groups (water and corn oil) whereas 7 mice in other groups. In total, 54 mice were used in the present study. Milli-Q water was used for dissolving PFOA and taurine, while corn oil was used for CoQ_{10}. All solutions were given to the animals orally (i.g.) at 10 ml/kg body weight (b.w.). PFOA was given in 15 and 30 mg/kg b.w. doses, which are below oral LD_{50} of mice (457 mg/kg) for 10 days [51]. Dose selection was based on previously published studies [52-55]. Taurine and CoQ_{10} were given to corresponding control groups at 100 mg/kg bw and 50 mg/kg bw, respectively, for 5 days. Doses selected according to earlier studies [56-60]. For evaluating the protective effects of both antioxidants, two animal groups were administered with corresponding antioxidant for 5 days followed by PFOA (30 mg/kg b.w.) treatment for 10 days. Experimental design for single PFOA and antioxidant + PFOA treatment groups was illustrated in Figure 7.

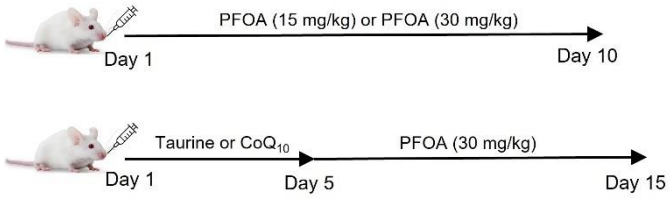

Figure 7. Experimental design for PFOA (15 and 30 mg/kg) and antioxidant + PFOA (30 mg/kg) treatment groups.

Following 24 h the last dose, mice were weighed and sacrificed through cervical dislocation using ketamine/xylazine anesthesia. Lung, heart and testis tissues were taken out, cleaned, rinsed with ice-cold 0.9% NaCl, blotted on filter paper and weights were measured in grams. Relative tissue weights were calculated as the ratio of tissue weight to body weight and given as percentage. Little portions of these tissues were fixed in 10% neutral formalin for histopathological evaluation. Other parts were kept at -80 °C for biochemical analysis.

5.3. Histological examination

Fixed tissue samples were dehydrated in gradient ethanol solution, then embedded in paraffin. They were subsequently portioned to 5-µm. After hematoxylin and eosin (H&E) staining, histopathological examination was made with a microscope at 200× magnification (Nikon Optiphot 2, Tokyo, Japan).

5.4. Sample collections

Tissues were homogenized with glass-glass homogenizer to achieve 10% (w/v) whole homogenate in 1.15% KCl. Portion of whole homogenate was employed to measure MDA level. Remaining parts were centrifuged at 2000 *g* for 10 minutes at 4°C to obtain supernatant. A portion of the supernatant underwent dilution with metaphosphoric acid (6%), followed by re-centrifugation at 2000 *g* for 10 minutes at 4°C, and obtained supernatant was used for total GSH analysis. Other part of the supernatant was re-centrifuged at 20,000 *g* for 25 minutes at 4°C, and antioxidant enzyme activities were measured by using resulting supernatant.

5.5. Oxidative stress markers and antioxidant enzyme activities

Lipid peroxidation was determined spectrophotometrically according to the method of Ohkawa et al. [61]. Thiobarbituric acid-reactive substances level was indicated as MDA nmol/mg protein. Measurement of total GSH concentration of tissues was made by method of Akerboom and Sies [62].

To determine Cu-Zn SOD activities of tissues, method of Arthur and Boyne [63] was used with slight changes. GPx activity was determined by method of Pleban et al. [64] and method of Aebi was used for the measurement of CAT activity [65]. Protein contents of tissue homogenates and supernatants were determined according to method of Lowry et al. [66].

5.6. Statistical analysis

The results were given as means ± SEM. Normality of data distribution was tested by Shapiro-Wilk test. Based on the results of this test, non-parametric Kruskal-Wallis test, followed by the Mann-Whitney *U* test, was employed for analysis of the statistical difference, using SPSS, version 18.0 (Chicago, IL, USA). $p < 0.05$ was deemed statistically significant.

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