Vitamin C Ameliorated Perfluorooctanoic Acid-Induced Thyroid and Testicular Dysfunction in Adult Male Rats.

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Abstract
This study aimed at investigating the ameliorative potentials of vitamin C in mitigating the negative consequences of long-term exposure to Perfluorooctanoic acid (PFOA) on the thyroid gland and testis of rats. Perfluorooctanoic is a member of widely used chemicals called Per- and polyfluoroalkyl substances (PFAS). For this purpose, a total of thirty mature male Wistar rats were divided randomly into five groups of equal size (6 rats per group): the normal control group, the sham group, the vitamin C control group, the PFOA treated group, and the PFOA-vitamin C treated group. Rats orally received 20 mg/kg of PFOA dissolved in Phosphate Buffer Saline for 60 days showed marked pathological alterations in thyroid and testis structure and functions. This reflected biochemically by significant decrease of thyroid hormones level, low free, total testosterone levels, significant increase of thyroid stimulating hormone (TSH), and estradiol levels in comparison with normal control group. The therapeutic group which orally received PFOA for 60 days then received 10 mg/kg of vitamin C dissolved in normal saline for 30 successive days showed marked improvement and regeneration of damaged thyroid and testicular tissues. This affirmed by histopathological findings of thyroid and testicular tissues. Overall, we recommend using vitamin C to mitigate the damage caused by long-term exposure to PFOA.

Keywords: PFAS. PFOA, Pathology, morphometry

Introduction
Per- and polyfluoroalkyl substances (PFAS) are a vast category of artificial chemicals that have been manufactured since the 1940s. They are known for their varied structures, properties, applications, ability to accumulate in living organisms, and potential for causing harm. [1, 2].

There are more than 4700 members in the PFAS family that may have been available worldwide. Despite their enormous diversity, all PFAS share a common characteristic: they possess a strong carbon-fluorine bond. This bond is what makes PFAS highly resistant to both environmental and metabolic destruction [2].

The molecular structure of PFAS contributes to their highly advantageous chemical and physical properties. The presence of a strongly fluorinated component in PFAS molecules renders them resistant to degradation via typical chemical, physical, or biological means, while also making them repel lipids and water. PFAS are valuable in industry due
to their high resistance to degradation, which allows them to be used in demanding conditions such as high temperatures, pressures, and corrosive environments. As a result, PFAS find applications in a diverse range of consumer, commercial, and industrial products, including adhesives, ceramics, cleaning products, coatings, wax, paint, inks, cosmetics, fire-fighting foam, and non-stick cookware. [3]. The exposure to PFAS can happen through several routes, such as contaminated food and water, work environments where PFAS are used, and everyday products containing these chemicals [4].

Perfluorooctanoic acid is mostly used member of PFAS as a surfactant and emulsifier linked to production processes for food packaging (like microwave popcorn bags) and non-stick cookware (Teflon), can surprisingly be found in everyday household items like clothes, carpets, and even paper [4]. Concerns about potential negative impacts on human health escalated in the early 2000s when considerable amounts of PFOA were found in human and animals’ blood [5].

Ingestion of polluted food and water, inhalation of dust with PFOA, and skin contact with PFOA-containing items are all potential routes for PFOA to enter the body. After being absorbed by the body, it has the ability to reach the blood stream, breast milk, liver, and kidneys, where it might accumulate. Animal studies demonstrate that following exposure to PFOA, the kidney exhibits the highest concentration, followed by the liver and lungs, which are the primary target organs. Perfluorooctanoic acid undergoes no metabolic processes within the body and is eliminated from the kidney without undergoing any changes and undergoes renal reabsorption [6]. In April 2024, the Environmental Protection Agency (EPA) settled a National Primary Drinking Water Regulation (NPDWR) establishing legally enforceable levels, called Maximum Contaminant Levels (MCLs) and health-based, non-enforceable Maximum Contaminant Level Goals (MCLGs), for PFOA in drinking water. The MCLG and MCL for PFOA is zero and 4.0 parts per trillion (ppt) (also expressed as ng/L) respectively [7].

Numerous negative consequences, including developmental toxicity, genotoxicity, carcinogenicity, hepatotoxicity, reproductive toxicity, immunotoxicity, cytotoxicity, neurotoxicity, and hormonal toxicity, are demonstrated by perfluorooctanoic acid [8]. Perfluorooctanoic acid is classified as an endocrine disrupting chemical (EDC) that has the potential to interfere with the functioning of the hypothalamic-pituitary-thyroid (HPT) axis and the action of thyroid hormones [9]. Moreover, it has been observed to induce hypertrophy or hyperplasia in thyroid follicular cells in rats, resulting in a reduction in both total and free thyroxine (T4) levels [9].

A study conducted on rats suggested that PFOA has a detrimental effect on the structure and function of the testis. The findings indicate that rats exposed to PFOA had a narrowing of the seminiferous tubule lumen and a thinning of the seminiferous epithelium. The organization of spermatogenic cells was disrupted, resulting in the shedding of a large number of immature spermatogenic cells into the lumen of the seminiferous tubule. Simultaneously, Sertoli cells and stromal cells exhibited vacuolation. Exposure to PFOA in male rats led to a considerable increase in levels of Follicle stimulating hormone (FSH), while levels of Testosterone (T) were lowered [10]. Additionally, serum estradiol (E2) levels
were found to be increased [11]. The binding of PFOA to estrogen receptors leads to the impairment of male reproductive function, oxidative damage to the body, disturbance of spermatogenesis through the induction of developmental impairment, and testicular oxidative stress [12].

On the other hand, Vitamin C, usually referred to as ascorbic acid, is a naturally occurring chemical molecule that possesses antioxidant capabilities. It is present in both animals and plants [13]. It is one of the potent reducing agents and acts by scavenging of reactive oxygen species (ROS) in biological systems [14]. Semen contains 65% of its antioxidant potential in the form of vitamin C, which is the most important antioxidant in semen. Studies have shown that it can effectively treat oxidative stress and sperm toxicity in rats [15]. Simultaneously, the consistent utilization of ascorbic acid has been found to elevate thyroid hormone levels because of its antioxidant characteristics. The administration of vitamin C safeguards against blood and thyroid toxicities caused by the prolonged usage of carbamazepine in rats [16].

**Materials and Methods**

**Ethical approval**

The procedures used in this experiment adhered to the ethical guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Zagazig University. Approval for this study was granted under committee permission number ZU-IACUC/2/F/446/2023.

**Drugs and chemicals**

Perfluorooctanoic acid, 96% purity, CAS No. 335–67-1 was purchased from Sigma-Aldrich (Saint Louis, USA) and ascorbic acid was purchased from the International Company for Scientific and Medical Supplies, Cairo, Egypt.

**Lab animals and Experimental protocol**

Thirty male albino Wistar rats, around 80-90 days old and weighing about 250 grams ± 30 grams, were purchased from the Laboratory Animal Unit at Zagazig University's Faculty of Veterinary Medicine in Egypt. A week prior to the experiment, 30 rats were housed in cages under controlled conditions: temperature at 23°C ± 2°C, 50% humidity, and a 12-hour light/dark cycle. The rats had unlimited access to food and water throughout this acclimation period. After this adjustment period, rats were randomly divided into five groups of six rats, each as follow:

1. Negative control groups

**Control group:** Received only standard laboratory chow and water.

**Vitamin C Control group:** Orally administered freshly prepared ascorbic acid (10 mg/kg body weight) dissolved in distilled water daily for 90 days [17].

**Sham group:** Orally administered 1 mL phosphate-buffered saline (PBS) daily via stomach tube for 90 days with standard laboratory chow and water.

2. Treatment groups

**PFOA:** Orally administered 20 mg PFOA/kg body weight of freshly prepared PFOA dissolved in PBS daily via stomach tube for 60 days [18].

**PFOA with Vitamin C:** Orally administered 20 mg /kg/day freshly prepared PFOA dissolved in PBS for 60 days followed by freshly prepared ascorbic acid (10 mg/kg body weight) dissolved in distilled water daily for 30 days.
During the 90-day experiment, the rats were carefully observed for any adverse clinical signs or deaths that may occur. All groups were euthanized on the 90th day of the experiment.

**Histopathological preparations**

Thyroid and testis specimens were obtained from all animals that were euthanized. The samples were immediately fixed in a solution consisting of 10% neutral buffered formalin. They were then dehydrated using a series of increasing concentrations of ethanol, cleaned using xylene, and finally embedded in paraffin. Sections with a thickness of 5 micrometers were prepared and stained with hematoxylin and eosin stain [17]. They were then examined under a microscope using an Olympus light microscope from Japan, which had an AmScope camera (MU500) attached to it. The images were recorded using AmScope 4.11 software from AmScope, USA.

**The immunohistochemical staining**

The formalin-fixed, paraffin-embedded testicular tissue samples from each animal were divided into consecutive sections. These sections were then stained for caspase3 using the Anti-Caspase-3 antibody [EPR18297] (ab184787, abcam, Inc.). The staining procedure was conducted utilizing the avidin-biotin-peroxidase complex technique [18]. The slides were subjected to treatment with 3,3'-Diaminobenzidine (DAB) to render the antigen-antibody complexes detectable. Subsequently, the nuclei were enumerated and stained with Mayer's hematoxylin stain. Five distinct, low-power microscopic fields (with 10 objectives) were acquired for each animal to quantify the proportions of brown DAB-stained region in relation to the total area of the images. The analysis was conducted using the ImageJ software, and the outcomes were expressed as percentages (mean ± SEM).

**The morphometric analysis**

Using AmScope v4.11 software, AmScope, Irvine, CA, USA, and the ImageJ software version 1.41 were utilized for the morphometrical analysis. The representative fields of H&E-stained sections at X 100 magnification were selected according to the presence of well-defined cross sections in the whole seminiferous tubules from each testis of the whole 6 animals/group to measure the diameter of seminiferous tubules and height of germinal epithelium. Furthermore, the diameter of thyroid follicles and area percentage of colloid inside thyroid follicles were calculated in 15 randomly selected thyroid follicles per animal and the area percentage of immune reactions to caspase3 was measured in caspase3-immunostained section at X400 magnification [19].

**Hormonal assay**

Blood samples were collected from the rats' tail vein. The levels of estradiol, free testosterone, total testosterone, Thyroid Stimulating Hormone (TSH), Thyroxine (T4), and Triiodothyronine (T3) in the serum were analyzed using specific ELISA kits designed for rats. These kits include the rat Estradiol (E2) ELISA Kit (Cat. No. MBS263466; MyBioSource, San Diego, CA, USA) (minimal detection level is 15.6 pg/mL), Rat Free Testosterone, F-TESTO ELISA Kit (Cat. No. MBS704301; MyBioSource) (minimal detection level is 0.3 pg/mL), Rat Testosterone, T ELISA Kit (Cat. No. MBS702057; MyBioSource) (minimal detection level is 0.13 ng/mL), rat (TSH) ELISA kit (Cat. No. MBS729687;
MyBioSource) (minimal detection level is 0.1 ng/mL), Rat thyroxine, T4 ELISA Kit (Cat. No. MBS9424770; MyBioSource) (minimal detection level is 20 ng/mL), and Rat Triiodothyronine (T3) ELISA Kit (Cat. No. MBS261285; MyBioSource) (minimal detection level is 10 ng/mL). The serum samples were treated according to the instructions provided by the manufacturers. Quantification of the results were done by exploiting a standard curve of a provided known concentration standard and absorbance (OD), finally, the slope of the curve was calculated, from which results were estimated according to supplier instructions.

Statistical analysis

The data were analyzed using GraphPad Prism 10.2 software (GraphPad Software Inc., San Diego, CA, USA). All results are presented as the average value (mean) with the standard error of the mean (SEM). To compare the groups, we performed a one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test. A p-value of less than ** P<0.01, *** P<0.001, and **** P<0.0001 was considered statistically significant.

Results

Histopathological findings

All subgroups of the negative control group (normal control, Sham and Vitamin C) were considered as one group as there were no differences between them in histopathological and immunohistochemical findings. Therefore, only the findings of the negative control were reported as a control group.

The histological observations of the thyroid gland in control, PFOA-treated, and PFOA with vitamin C co-treated rats.

1. Negative control group

Examination of the thyroid gland in the control group revealed normal architecture. The follicles were divided into numerous lobules and varied in size. These follicles were filled with a homogeneous, acidophilic colloid (Figure 1A).

2. PFOA-Treated Group

In contrast, the PFOA-treated group displayed significant alterations in thyroid morphology. Several central follicles exhibited signs of degeneration, lacking colloid entirely. Peripheral follicles within the same section might be partially filled with colloid (Figure 1B). Degenerative changes were also evident in follicular cells adjacent to dilated follicles. These affected cells showed hydropic degeneration and were positioned near follicles containing either dense or absent colloid (Figure 1C). Calcium oxalate crystals were additionally observed within the colloid of some degenerated follicles (Figure 1D). Furthermore, some follicular cells displayed signs of necrosis, characterized by pyknosis and karyolysis (Figure 1E). Desquamated follicular cells within the lumen, congested blood vessels, and widened interfollicular spaces were also observed in the PFOA-treated group (Figure 1F).

3. PFOA with Vitamin C Co-Treated Group

The thyroid gland in the PFOA with vitamin C co-treated group displayed a mixed picture. While some follicles appeared similar to the control group, others exhibited peripheral vacuolation within the colloid (Figure 1H). Hyperplasia, characterized by multiple layers of follicular cells, was observed in some sections alongside congested blood vessels (Figures 1 I and J).
Figure 1. Figure (1): photomicrograph of thyroid gland sections of: (A) control rat showing different sizes of thyroid follicles and the follicular lumen filled with colloid (blue asterisk) large peripheral follicles (blue arrow) and small central one (black arrow). (B) Thyroid gland of PFOA treated group showing degenerated central follicles without colloid (black arrow) and the peripheral one partially filled with colloid (blue arrow). (C) Degenerated follicle with hydropic degeneration (green arrow) adjacent to dilated follicles that contain dense colloid (black arrow) or free from colloid (blue arrow). (D) Calcium oxalate crystals within the colloid of some degenerated follicles (black arrow). (E) Some follicular cells showing massive vacuolation (red arrow) while other showing pyknosis (blue arrow) and karyolysis (green arrow). (F) Disorganized thyroid follicles with desquamated follicular cells in the follicular lumen (green arrow), congestion of blood vessels (black arrow) and wide interfollicular space (blue arrow). (G) High power of previous figure to show disintegrated follicles (red arrow) and desquamated follicular epithelium (green arrow). (H) Thyroid gland of treated group (PFOA+Vit C) showing most of follicles filled with peripheral vacuolated colloid (red arrow). (I) Hyperplasia of follicular cells represented by multiple layers of follicular cells (black arrow) with congested blood vessels (red arrow) and peripheral vacuolated colloid (blue arrow). (J) High power of previous figure to show hyperplasia of follicular cells represented by multiple layers of follicular cells (black arrow), Scale bar for (A, B, F, H and I) = 100 μm, Scale bar for (C, D, E, G and J) = 20 μm. H&E stain.

The histological changes observed in the testes of control, PFOA-treated, and PFOA with vitamin C co-treated rats

1. Negative control group

Examination of the testes in negative control group revealed a normal histological appearance. The seminiferous tubules were well-organized, with various stages of spermatogenic cells lining their walls (spermatogonia, spermatocytes, spermatids, and spermatozoa). Additionally, normal interstitial tissue containing Leydig cells and blood vessels was observed (Figures 2A and B).

2. PFOA-Treated Group

Exposure to PFOA resulted in significant testicular damage. Degenerative and necrotic changes were observed in spermatogenic cells within some seminiferous tubules. These affected tubules displayed spermatogenic arrest at the spermatocyte stage, indicating disrupted spermatogenesis (Figure 2C). Further evidence of damage included desquamated spermatogenic cells, hyaline material within the lumen, vacuolation of germinal cells, and fibrosis of the interstitial tissue (Figures 2D and E). In some cases, complete necrosis of all spermatogenic cells was observed, with no evidence of ongoing spermatogenesis and increased interstitial space (Figure 2F).

3. PFOA with Vitamin C Co-Treated Group

Vitamin C co-treatment appeared to offer some protective effects on the testes compared to PFOA alone. While some seminiferous tubules displayed mild
vacuolation of the germinal epithelium and interstitial edema (Figure 2G), the overall damage was less severe than in the PFOA-only group. Additionally, some tubules showed evidence of regeneration, with active spermatogenesis displacing degenerated areas and congested blood vessels (Figure 2J). Interestingly, some degenerated tubules displayed a wrinkled basement membrane and multiple intratubular spermatid giant cells (Figure 2I). Notably, most seminiferous tubules within the co-treated group exhibited a more normal morphology compared to PFOA alone, with spermatogenic cells arranged in well-defined layers within tubules of a more regular shape, suggesting a degree of restoration of spermatogenesis.

The Immunohistochemical findings

The Immunohistochemical staining for caspase-3 expression was seen in (Figure 3). The testicular tissues of the control and vitamin C-treated rats revealed negative expression for caspase-3 (Figure 3A) and mild immunoreactivity in PFOA-treated rats (Figures 3B and C). Moreover, weak immunoreactivity in the PFOA-vitamin C treated rats (Figure 3D).
**Morphometric analysis**

Morphometric analysis of thyroid of rats from PFOA treated group showed significant (P<0.01) decrease in thyroid follicles diameter (Figure 4 A), significant (P<0.001) decrease in % area of colloid (Figure 4 B) inside thyroid follicles, significant (P<0.0001) decrease in seminiferous tubular diameter (Figure 4 C) and significant (P<0.001) decrease in seminiferous epithelium height(Figure 4 D) in comparison with normal control, Sham and Vitamin C and PFOA- vitamin C treated rats.
Hormonal assay findings

This section explores the impact of PFOA and vitamin C co-treatment on hormone levels in rats:

1. PFOA-Treated Group:

Compared to the control groups, PFOA exposure resulted in significant (p<0.001) decreases in circulating levels of triiodothyronine (T3) (Figure 5A), thyroxine (T4) (Figure 5B), free testosterone (Figure 5D), and total testosterone (Figure 5E). Conversely, PFOA treatment caused a significant (p<0.001) increase in thyroid-stimulating hormone (TSH) (Figure 5C) and estradiol (Figure 5F) levels.

2. PFOA with Vitamin C Co-Treated Group:

Importantly, vitamin C co-administration significantly (p<0.001) improved the hormonal profile in PFOA-treated rats.

Overall, these findings suggest that PFOA disrupts the hormonal balance, leading to decreased levels of thyroid hormones and sex hormones, while increasing TSH and estradiol. Vitamin C co-treatment appears to offer significant protection against these hormonal disruptions caused by PFOA exposure.
Perfluorooctanoic acid is a newly discovered organic environmental pollutant distributed in nature due to the extensive use that raised regarding their potential risk to humans and animals [20]. This study is an approach to find whether vitamin C can treat the lesions induced by PFOA in rats.

Thyroid sections from PFOA treated group revealed some degenerated central follicles without colloid and the peripheral one partially filled with colloid. Some follicular cells showed hydropic degeneration adjacent to diluted follicles that contain dense colloid or free from colloid. Additionally, calcium oxalate crystals within the colloid of some degenerated follicles were also seen. Furthermore, some follicular cells revealed necrosis represented by pyknosis and karyolysis. Desquamated follicular cells in the follicular lumen, and wide interfolllicular space were also observed. The previous findings are in a partial concurrence with Manera et al. [21] who observed that, PFOA has been found to have various impacts on the structure and ultrastructure of the thyroid. The exposure to 2 mg L⁻¹ PFOA resulted in notable alterations in thyroid follicles, including an augmentation in follicle abundance and vesiculation. The presence of vesiculation suggests hyperactivity and/or early degeneration, which was particularly visible in the fish exposed to this concentration [22].

PFAS have been observed to induce hypertrophy or hyperplasia of thyroid follicular cells in rats and have been linked to decreased levels of total and free thyroxine (T4) concentrations [22].
The thyroid system may be negatively affected by PFOA due to its ability to alter the structure of the epithelium and/or colloid, as well as its cytotoxic effects on thyroid cells. PFOA can also impact the function of receptors, transport proteins, and enzymes that are involved in the metabolism of thyroid hormones [23].

The reduced concentration of thyroid hormones was induced by elevated levels of reactive oxygen species (ROS), leading to the impairment of thyroid follicular cells (the primary sites of thyroid hormone synthesis) [24]. TSH is responsible for the morphological appearance of thyroid follicles [25]. Furthermore, most of the prior alterations in the structure of the thyroid follicles may have been a result of excessive secretion of TSH, which was an attempt to counterbalance the decrease in T3 and T4 levels. Moreover, the observed follicular colloid vacuolation can be attributed to the elevated TSH level. Literature suggests that under conditions of high thyroid hormone demand, follicular cells may extend pseudopods into the lumen. These finger-like projections engulf and resorb colloid, leading to the characteristic vacuolated appearance [26].

The PFOA+vitamin C treated group exhibited partial restoration of normal thyroid architecture. The majority of follicles displayed peripherally vacuolated colloid, potentially indicative of increased resorption activity. Additionally, some sections revealed follicular cell hyperplasia, characterized by multiple cell layers and congested blood vessels. However, a subset of follicles appeared histologically similar to the control group.

The current study revealed that treatment with PFOA for 60 consecutive days resulted in obvious morphological alterations in the testis of rats, including congested blood vessels adjacent to degenerative and necrotic spermatogenic cells with spermatogenic arrest at spermatocyte stage. Some seminiferous tubules showed desquamated spermatogenic cells and hyalinized material inside lumen with vacuolation of germinal cells and fibrosis of interstitial tissue. Most seminiferous tubules revealed severe necrosis of all spermatogenic cells with no evidence of spermatogenesis and increase interstitial space. The obtained outcomes partially align with the findings of Yuan et al. [27] which demonstrated that mice, when orally given a daily treatment of 10 mg per kg of PFOA for 21 consecutive days, experienced seminiferous tubule atrophy, disarray of seminiferous epithelium, lack of spermatozoa, reduction of spermatogonial cells, and detachment of germ cells. Reproductive harm caused by PFOA in rats administered 0.01 g/kg of PFOA through gavage for a duration of 30 days [10]. However, current rodent research shows that Leydig cell [28], Sertoli cells [29], and sperm [30] are targets of PFOA. Moreover, Zhang et al., [28] reported that chronic exposure to PFOA could induce damage the structure of the testis. Meanwhile, our results are in agreement with Cui et al. [31] who showed no distinct pathological change was found in the testicles exposed to 5-20mg/kg PFOA/ day.

Our findings align with previous studies suggesting that PFOA-induced testicular damage may be mediated by oxidative stress and apoptosis [12,26]. Exposure to PFOA in other models has been linked to excessive ROS production and increased apoptosis in the testes [27]. When the testes encounter harmful substances like PFOA, they may generate excessive amounts of reactive oxygen species (ROS) [32]. If the production of ROS overwhelms the antioxidant defenses...
within the testes, oxidative stress can occur [33]. Notably, a recent study demonstrated that PFOA can negatively impact sperm quality by inducing oxidative stress and triggering an influx of calcium from outside the cell, ultimately leading to a higher percentage of abnormal sperm [30].

Our own observations support these prior findings. The PFOA-treated group displayed histological evidence of testicular injury, which could be a consequence of the observed alterations. Further investigation is needed to directly assess markers of oxidative stress and apoptosis in our PFOA-exposed animals.

As mentioned before PFOA had a great effect on thyroid, as known Thyroid hormones have a crucial role in the development and function of reproductive system. Hypothyroidism seems to have a greater impact on the function of Leydig cells than on Sertoli cells [34]. This may be the cause of our finding of low testosterone levels. Another cause for low testosterone levels is the impact of PFOA on aromatase activity. PFOA increases the activity of aromatase resulting in increased level of estrogen level [35].

On the other hand, treatment of rats with vitamin C for 30 consecutive days after administration of PFOA ameliorate the previous lesions induced by PFOA. Most seminiferous tubules of this group showed regeneration of germinal epithelium and displacing of active spermatogenesis. The shapes of seminiferous tubules became more regular, and the spermatogenic cells were closely arranged with distinct layers spermatogenic cells. Hemorrhage among degenerated seminiferous tubules was seen in some seminiferous tubules. The previous findings were nearly similar to those mentioned by Li et al. [36] who observed that, Vitamin C attenuated the reduction of seminiferous tubular diameters probably because this vitamin may have acted in the synthesis of extracellular matrix components. Vitamin C’s potential curative effect may be due to its antioxidant properties. Literature suggests it protects sperm and Leydig cells (responsible for testosterone production) from oxidative damage [37]. Vitamin C constitutes a major portion (65%) of the semen's antioxidant capacity and has been shown to alleviate oxidative stress and sperm toxicity in rats [15]. Additionally, it plays a role in testosterone synthesis [38]. The partial recovery of LH levels in the vitamin C treated group might be linked to its potential role as a "vitaminergic transmitter," stimulating the release of LH and FSH (hormones crucial for sperm production) from the pituitary gland.

The Immunohistochemical staining for caspase-3 expression in the testicular tissues of the control and vitamin C-treated rats revealed negative expression and mild immunoreactivity in PFOA-treated rats and weak immunoreactivity in the PFOA-vitamin C treated rats [39]. Apoptosis is a natural biological process that involves the targeted elimination of specific cells. Apoptosis, which acts as an inhibitor of cell proliferation, has a role in regulating the number of cells in testicular tissue and eliminating unnecessary or damaged cells. However, excessive apoptosis can lead to the impairment of male reproductive function [40]. The liberation of PFOA-derived free radicals and reactive species led to the breaking down of DNA, enhanced the release of cytochrome C from the mitochondria to the cytosol, and raised pro-apoptotic markers, resulting in an elevated occurrence of apoptosis. These results indicate that Vitamin C successfully reversed the structural and functional harm caused by PFOA to the
thyroid gland. It is widely recognized that stem cells residing in tissues remain inactive and are stimulated to become active and divide in response to injury [41].

Vitamin C treatment may have mitigated the inhibitory effect of PFOA on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity. These two key antioxidant enzymes are essential for scavenging and neutralizing harmful reactive oxygen species (ROS) within the cell [42]. With subsequent relieve of the state of oxidative stress and improvement in the morphology of the thyroid gland.

Conclusion

The findings of the current study declared that the use of vitamin C amended perfluorooctanoic acid -induced thyroid and testicular dysfunction in rats and in turn we indorse using vitamin C to moderate the damage caused by long-term exposure to PFOA.

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Conflicts of Interest:

The authors declare no conflict of interest.

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الملخص العربي

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هذه الدراسة تهدف إلى استكشاف القدرات التحسينية لفيتامين سي في تخفيف الآثار الضارة لل تعرض طويل الأمد لحمض البيرفلوروكتانويك على الغدة الدرقية والخصية لدى الفئران. حمض البيرفلوروكتانويك هو عضو في مجموعة واسعة من المواد الكيميائية تسمى مواد البير والبولي فلوروالكايلز. لتحقيق هذه الغاية، تم تقسيم إجمالي ثلاثين فأر من نوع ويستار عشوائياً إلى خمس مجموعات متساوية الحجم (ست فئران لكل مجموعة): مجموعة التحكم الطبيعية، ومجموعة الظاهرية، ومجموعة التحكم بفيتامين سي، ومجموعة المعالجة بحمض البيرفلوروكتانويك، ومجموعة المعالجة بحمض البيرفلوروكتانويك وفيتامين سي. أظهرت الفئران التي تلقت 20 ملمغ / كجم من حمض البيرفلوروكتانويك المذاب في محلول ملحي فوسفاتي متعادل عن طريق الفم لمدة 60 يومًا تغيرات مرضية ملحوظة في بنية ووظائف الغدة الدرقية والخصية. انعكس هذا كيميائيًا بالانخفاض ملحوظ في مستويات هرمون ترسيبون الحر والكلي، وزيادة ملحوظة في مستويات هورمونات الغدة الدرقية والخصية ومستويات التترسترويين. أظهرت المجموعة المعالجة التي تلقت حمض البيرفلوروكتانويك لمدة 60 يومًا تقلصًا كبيرًا في كجم من فيتامين سي المذاب في محلول ملحي عادي عن طريق الفم لمدة 30 يومًا مثلاً صاحبًا ملحوظًا وتجدًا للأنسجة التالفة في الغدة الدرقية والخصية، وهو ما أظهره النتائج السنجية المرضية للنسبة الغدة الدرقية والخصية. بشكل عام، نوصي باستخدام فيتامين سي للتخفيف من تلف الإجهاد التأكسدي الناجم عن التعرض طويل الأمد لحمض البيرفلوروكتانويك.