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# Impact of variations in oral and intraperitoneal administration of cyclophosphamide on testicular toxicity in adult male albino rats

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**ABSTRACT**

Cyclophosphamide (CTX) is commonly used as chemotherapeutic and immunosuppressive agents. Despite its potential effectiveness treatment, it has adverse effects on the structure and functions of various body organs. This study was conducted to address testicular dysfunctions in the testes post CTX-treatments either with intraperitoneal injection (i.p.) or with oral administration (P.O.). Thirty male rats were used, and were divided into three groups (10 rats for each) as follows: Group 1 (Gp1) was served as control group. Gp2, rats had given a single dose of CTX (200 mg/kg b.wt) i.p. Gp3, rats were given a single dose of CTX (200 mg/kg b.wt) P.O. Rats were sacrificied at day 8. Blood samples were collected and the testes were removed for histological examination. The antioxidant/oxidant parameters, male reproductive hormones included testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), pro-inflammatory cytokines included tumor necrotic factors-α, and inteleukin-6 were measured. The histological alterations in testes in all groups were investigated. CTX-i.p. group demonstrated highly significant decrease in superoxide dismutase activity, catalase activity and glutathione reduced content while mild significant reduction observed in CTX-P.O. group when compared to control group. Malondialdehyde level showed highly significant increase in CTX-i.p. and moderate significant increase in CTX-P.O. group when compared to control group. The results showed highly significant reduction in hormone levels (testosterone, LH and FSH) in CTX-i.p. group when compared to control group while CTX-P.O. group showed moderate significant decrease in hormone levels when compared to control group. The results showed that CTX-i.p. injected group triggers a strong inflammatory response than CTX-P.O. group when compared to control group. The histological examinations of testes tissues confirmed these results. The CTX injection through the i.p. injection led to more toxicity in the physiological and histological alteration in testes when compared to the administration of CTX by P.O.

**Keywords:** Cyclophosphamide, Inflammation, Oxidative stress, Sexual hormones, Testes

## 1. Introduction

Cyclophosphamide (CTX) is a commonly used immunosuppressive and chemotherapeutic drug used to treat a variety of malignancies. Active metabolites such as phosphoramide mustard and acrolein are produced from CTX, a prodrug, by liver cytochrome P450 enzymes (Dabbish et al., 2024). The alkylation of DNA by these metabolites results in strand breakage and cross-linking, which trigger death in cells that are proliferating (Zhang et al., 2025). According to Sheet (2024), these pharmaceuticals, like other chemotherapeutic drugs, may be linked to numerous adverse effects. Some individuals using this therapy may experience modest side effects such as gastritis, alopecia, and reduced immunity, while others may encounter severe

adverse effects including pulmonary fibrosis, hepatotoxicity, and cardio toxicity. Cyclophosphamide, similar to numerous other chemotherapeutic agents, can impact fertility and cause reproductive toxicity, which can result in infertility in both sexes (Abdi et al., 2024). Thrombocytopenia, leukopenia, and anemia were symptoms of bone marrow suppression due to CTX toxicity. Because of these negative effects on quickly dividing cells, blood counts must be regularly monitored throughout treatment (Yan et al., 2024). According to Thompson et al. (2023), prolonged usage of CTX raises the risk of developing secondary cancers, including acute myeloid leukemia and bladder cancer. In addition to its cytotoxic effects, low-dose CTX enhances the body's anti-tumor immune response by specifically reducing regulatory T cells (Bakar and Kue, 2024). The efficacy of CTX in certain patients may be limited by increased glutathione pathway activation, elevated aldehyde dehydrogenase activity, and improved DNA repair mechanisms (Cesca et al., 2025). In order to decrease toxicity and improve antiangiogenic effects, metronomic dose of CTX was investigated in a prior study (Jan et al., 2024).

Oral administration (P.O.) of CTX is associated with variable absorption due to factors such as food intake, gut microbiota, and first-pass hepatic metabolism. This can lead to inter-patient variability in drug exposure (Khorashadizadeh et al., 2024). In contrast, i.p. injection in animal models tends to provide more consistent absorption and higher systemic availability (Chen et al., 2024).

The route of administration may influence antitumor efficacy, particularly in localized disease models. The i.p. injection can result in higher peritoneal cavity drug concentrations, offering greater efficacy in treating peritoneal carcinomatosis or ovarian cancer models (Fu et al., 2017). Because the i.p. absorbs and reaches peak plasma levels more quickly, it may cause more acute systemic toxicities, particularly when given in high doses. In contrast to P.O., studies conducted on mice have demonstrated that i.p. can cause more severe immunosuppression and gastrointestinal damage (Vlastou et al., 2025). Since localized drug distribution is beneficial, i.p. is mostly restricted to experimental models or certain intraperitoneal cancers. But when given at the right dosage, the P.O. administration of CTX for systemic tumors has shown similar effectiveness in clinical settings (Mir et al., 2024). P.O. may be linked to gastrointestinal distress and an increased risk of bladder toxicity because of extended exposure to metabolites, while being less intrusive and more convenient (Thompson et al., 2023). Clinically speaking, P.O. is preferred since it is simple to use and comfortable for patients, especially when used in low-dose or long-term metronomic regimens. Peripheral blood cells decreased as a result of myelosuppression brought on by CTX medication. The metabolic activity of the cytochrome P450 mixed functional oxidase system caused CTX to produce two different metabolites: phosphoramide mustard and acrolein (El-Naggar et al., 2017). These metabolites trigger the oxidative stress believed to be the main cause of CTX toxicity. CTX damages proteins, DNA, and lipids in testicular tissue via generating reactive oxygen species (ROS) during metabolism. This oxidative damage leads to Leydig and germ cell death, mitochondrial dysfunction, and lipid peroxidation (Naghdi et al., 2016). Less testosterone is produced as a result of CTX's impairment of Leydig cell activity. Testicular homeostasis and spermatogenesis are further disturbed by this hormonal imbalance (Jiang et al., 2025). In the testes, CTX dramatically raises germ cell apoptosis. Increased DNA fragmentation in spermatogenic cells in animal models, suggesting apoptosis as a major mechanism of testicular toxicity (Gungor-Ordueri et al., 2021). The cumulative damage leads to reduced sperm count, poor motility, and abnormal morphology, significantly compromising fertility. These effects may be dose-dependent and, in some cases, irreversible depending on exposure duration and intensity (Jiang et al., 2024).

Because CTX is toxic to Leydig cells, which produce testosterone in the testes, it significantly lowers testosterone levels, and disturbed spermatogenesis (Chavaengkiat et al., 2024). ROS-induced Leydig cell death is one of the reasons for hormonal disturbance (Abarikwu et al., 2012), direct DNA damage to steroidogenic tissues, and inflammation with altered enzyme

activity in testosterone biosynthesis pathways (Naghdi et al., 2016). Therefore, the current study was conducted to compare the effect of given CTX by i.p. or P.O on some physiological and histological parameters of rat's testes.

## 2. Materials and methods

### Chemicals

CTX was purchased from Sigma–Aldrich Chemicals Co., St. Louis, MO, USA. CTX was dissolved in normal saline for i.p. to the experimental rats.

### Animals

The study was performed on 30 healthy Wister adult male albino rats (weighted from 125 to 135 g, $9^{th}$ – $10^{th}$ weeks of age). Rats were purchased from the National Research Centre (NRC) in Giza, Egypt, and housed in well-ventilated cages, at room temperature 26 ± 2°C and humidity of 58 ± 5% under 12 hr dark-light cycle for 2 weeks before the experiment. The experimental protocols follow the Guidelines used for Animal Experimentation and approved by the ethical committee of the Faculty of Science at Damanhur University, Egypt DMU-SCI-CSRE-25-04-08. Animals were fed standard diet with free access to water *ad libitum*. Animals were carefully observed every day and their body weights, while food consumption and water intakes were measured precisely every week to evaluate any signs of toxicity or abnormality during the experiment.

### Experimental design

The experimental rats were randomly divided into the three groups (10 rats for each) as follows: group 1 (Gp1) was served as control group. Gp2, rats had given a single dose of CTX (200 mg/kg b.wt) through intraperietoneal (i.p.) injection. Gp3, rats had administered with a single dose of CTX (200 mg/kg b.wt) oral administration (P.O.) (Alsemeh and Abdullah, 2022).

The experimental protocol concluded with all rats being euthanized after 24 hours of fasting, i.p. injection with sodium pentobarbital, and exposed to a complete necropsy. Blood samples from rats were collected in non-herpanized, dry and clean glass test tubes. Centrifugation of the specimens at 1922 Xg for 15 minutes at 4°C was used to separate the serum.

The testes were removed from the dissected rats and separated into two equal portions. Ice-cold saline solution was used to wash each portion. Left testes was preserved in Bouin′s solution for histological examination, while the right testes and the serum samples were kept at –80°C until they were needed for biochemical analyses.

### Biochemical analysis

### Preparation of testis tissue homogenate

The tissue homogenate of testis was prepared. To obtain 10% (w/v) homogenates, one gram of tissue was cut into small pieces and submerged in ice-cold 0.1M phosphate buffer (PBS, pH 7.4). Then the homogenates were centrifuged at 4°C for 15 minutes at 1922 Xg, and the supernatants were separated and stored at –80°C until use.

### Determination of testicular antioxidants and oxidative stress

Superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and malondialdehyde (MDA) kits were purchased from Biodiagnostic Company, Egypt. The SOD activity was estimated according to the method of Nishikimi et al. (1972). GSH content and CAT activity were calculated in accordance with Meister (1988) and Aebi (1984), respectively. Level of MDA was estimated according to Kei (1978).

### Determination of serum hormone profile

Serum testosterone, luteinizing hormone (LH), and follicular stimulating hormone (FSH) levels were measured by using Rat ELISA kit from Cusabio Co. (Fannin, Houaton, TX, USA) with catalog numbers: CSB-E05097r, CSB-E12654r, and CSB-E06869r, respectively.

### Determination of testicular proinflammatory cytokines

Determination of tumor necrosis factor - α (TNF-α) and interleukin-6 (IL-6) were determined by using Rat ELISA kits from Cusabio Co. (Fannin, Houaton, TX, USA) with catalog numbers: CSB-E11987r and CSB-E04640r, respectively.

### Histopathological investigations

The tiny testicular tissue fragments were promptly preserved for a full day in Bouin′s solution. The tissue samples were cleaned to get rid of extra fixative, and then dehydrated in ethyl alcohol grades that went up, cleaned with xylene,

and then embedded in paraffin wax. Hematoxylin and eosin staining was applied to sections 5um thick for histological analysis (Tawfiek, 2016).

**Statistical analysis**

All data are the means of three replicates. one-way analysis of variance (ANOVA) was used, if there is significant differences between means, Tukey's *post-hoc* comparisons among different groups were performed. *p*- values< 0.05 considered significance. Graph Pad Prism V. 8.3, IBM SPSS Statistics for Windows, Version 27, and Microsoft Excel 365 (Microsoft Corporation, USA) were used to analyze all of the data.

## 3. Results

**Changes in antioxidants and oxidative stress**

Figure (1), showed the effects of CTX administered *via* P.O. and i.p. injection on oxidative stress markers in testicular tissues. The biomarkers evaluated include SOD, CAT, GSH, and MDA. The results showed that the SOD activity was highest in the control group (9.47 ± 0.15 U/g tissue), and highly significantly decreased in the CTX-i.p. group (4.37 ± 0.4), indicating pronounced oxidative stress. The CTX-P.O. group (8.63 ± 0.21) showed a moderate significant decrease. CAT activates followed a similar trend. Control group had the highest CAT activity (25.07 ± 0.38 U/g tissue), while CAT had highly significant decline in the CTX-i.p. group (13.1 ± 0.44), while a less severe reduction was observed in the CTX- P.O. group (19.3 ± 0.3). GSH concentration was highly significantly decreased in the CTX-i.p. group (17.63 ± 0.15 mM/g tissue) compared to control (33.6 ± 0.26), while the P.O. group showed substantially decreased levels (29.4 ± 0.2). This suggests that i.p. injection causes significant reduction of antioxidant enzymes. As a marker of lipid peroxidation, MDA levels were significantly increased in the CTX-i.p. injected group (19.93 ± 0.51 nmol/g tissue), confirming oxidative tissue damage. The CTX-P.O. group (14.5 ± 0.2) showed a moderate significant but highly significant increase, respectively compared to control (13.23 ± 0.25). (Figure 1).

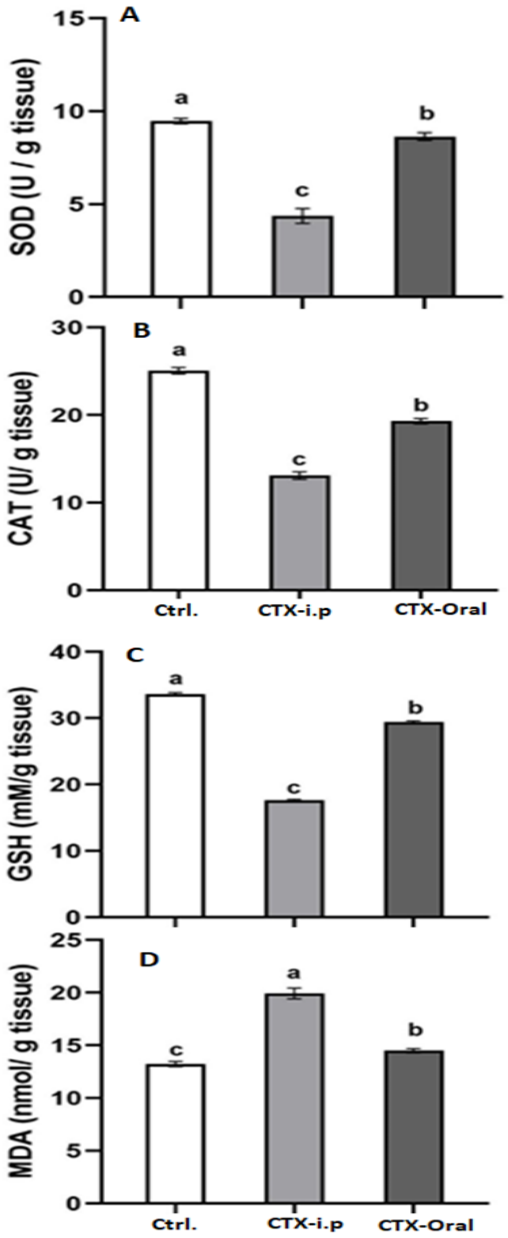


**Fig. 1 (A-D).** Testicular activities of SOD (A) and CAT (B), the content of GSH (C), and the level of MDA in the different experimental groups. The data expressed as (mean ± SD) at ($p$< 0.05). Superscript letters (a, b, and c) represented that groups sharing the same letter are not significantly different from each other, while different letter indicate statistically significant differences. Control group (Ctrl.), cyclophosphamide intraperitoneal injected group (i.p.) (CTX- i.p), and, cyclophosphamide oral administered group (P.O.) (CTX- P.O).

**Changes in the level of reproductive hormones**

The results presented in Figure (2) examined the effects of P.O administration and i.p. injection of CTX on hormone levels, specifically testosterone, LH, and FSH in rats. Control group exhibited the highest levels of all three hormones (testosterone: 3.36 ± 0.21 mIU/ml, LH: 3.45 ± 0.08 ng/ml and FSH: 1.84 ± 0.05 mIU/ml). CTX-

i.p. injection group showed highly significant reduction in hormone levels (testosterone: 0.99 ± 0.05 mIU/ml, LH: 1.07 ± 0.03 ng/ml and FSH: 0.70 ± 0.04 mIU/ml) when compared to the control. CTX-P.O. administration group had moderate significant decrease in hormone levels compared to the control (testosterone: 1.72 ± 0.06 mIU/ml, LH: 2.73 ± 0.09 ng/ml and FSH: 1.31 ± 0.1 mIU/ml).

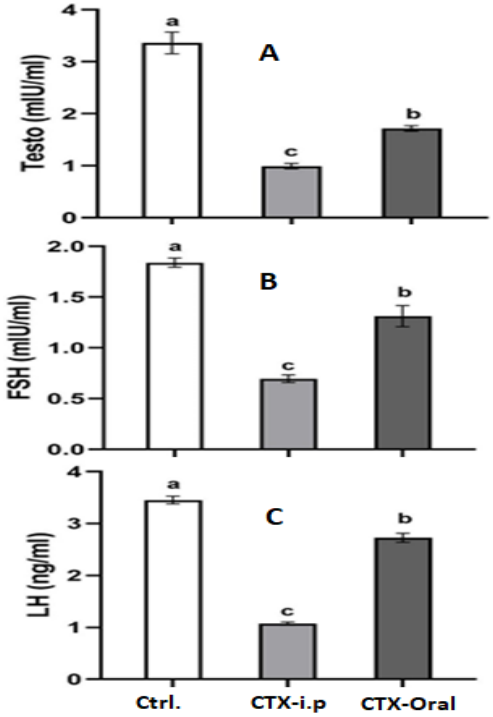


**Fig. 2 (A-C).** Serum levels of testosterone (A), FSH (B), and LH (C) in the different experimental groups of rats. The data expressed as (Mean ± SD) at ($p$ < 0.05). Superscript letters (a, b, and c) represented that groups sharing the same letter are not significantly different from each other, while different letter indicate statistically significant differences. Control group (Ctrl.), cyclophosphamide intraperitoneal injected group (i.p.) (CTX-i.p), and, cyclophosphamide oral administered group (P.O.) (CTX-Oral).

## Changes in the content of pro-inflammatory cytokines

Figure (3), presented the effects of CTX *via* P.O. and i.p. on pro-inflammatory cytokines, TNF-α and IL-6, in testicular tissue of rats. These biomarkers are critical for evaluating inflammation-related damage in reproductive tissues. TNF-α level were moderate significantly increased in the CTX-i.p. group (67.5 ± 0.52 Pg/g tissue), compared to control (46.67 ± 0.25) and insignificant changes in CTX-P.O. group (47.47 ± 0.38). This suggests that the i.p. injection of CTX triggers a strong inflammatory response. IL-6 levels were highly significant increase in CTX-injected rats (8.47 ± 0.12 Pg/g), and a moderate significant increase in group of rats that has CTX through the P.O. administration (6.17 ± 0.15) compared to the control group (5.8 ± 0.1). This indicates enhanced pro-inflammatory signaling, especially following i.p. injection (Figure 3).

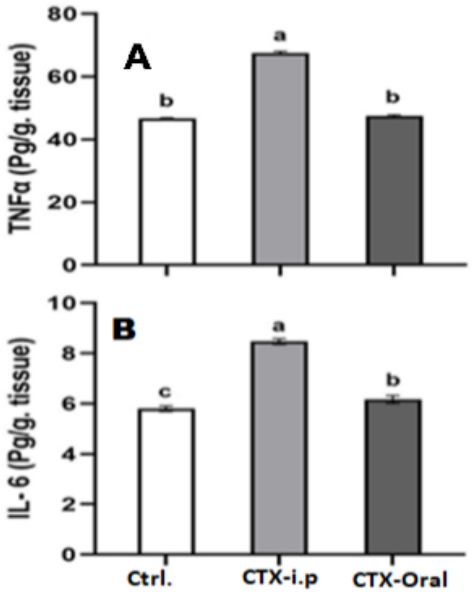


**Fig. 3 (A-C).** (A). the testicular pro-inflammatory cytokines level including TNF-α (A) and IL-6 (B) in the different experimental groups of rats. The data expressed as (mean ± SD) at ($p$< 0.05). Superscript letters (a, b, and c) represented that groups sharing the same letter are not significantly different from each other, while different letter indicate statistically significant differences. Control group (Ctrl.), cyclophosphamide intraperitoneal injected group (i.p.) (CTX-i.p), and, cyclophosphamide oral administered group (P.O.) (CTX-Oral).

## The histopathological investigations

Microscopic examination of testis sections of control exhibits many seminiferous tubules that are surrounded by a thin, regular basement membrane. Each seminiferous tubule is lined with stratified germinal epithelium, including spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids with intraluminal spermatozoa. Leydig cells are found in the thin interstitium that separates semi-niferous tubules. (Gp2) CTX-i.p. group exhibit severe damage which has deformed basement membranes and distorted seminiferous tubules

with marked reduction in germinal epithelium thickness. These tubules contain cells that are highly degenerated, having vacuolated cytoplasm and pyknotic nuclei. The lumen of seminiferous tubules displays sloughed germinal cells and is devoid of spermatozoa. The interstitial space is wide and contains degenerated Leydig cells.

Seminiferous tubules in the CTX-P.O. administered group (Gp3) revealed a considerable decrease in the thickness of the germinal epithelium. These tubules include extremely degenerated cells with pyknotic nuclei and vacuolated cytoplasm, wide spaces between germ cells. Seminiferous tubules display edema in the lumen and lack spermatozoa, while edema and vacuolated Leydig cells can be observed in interstitial space. (Figure 4).

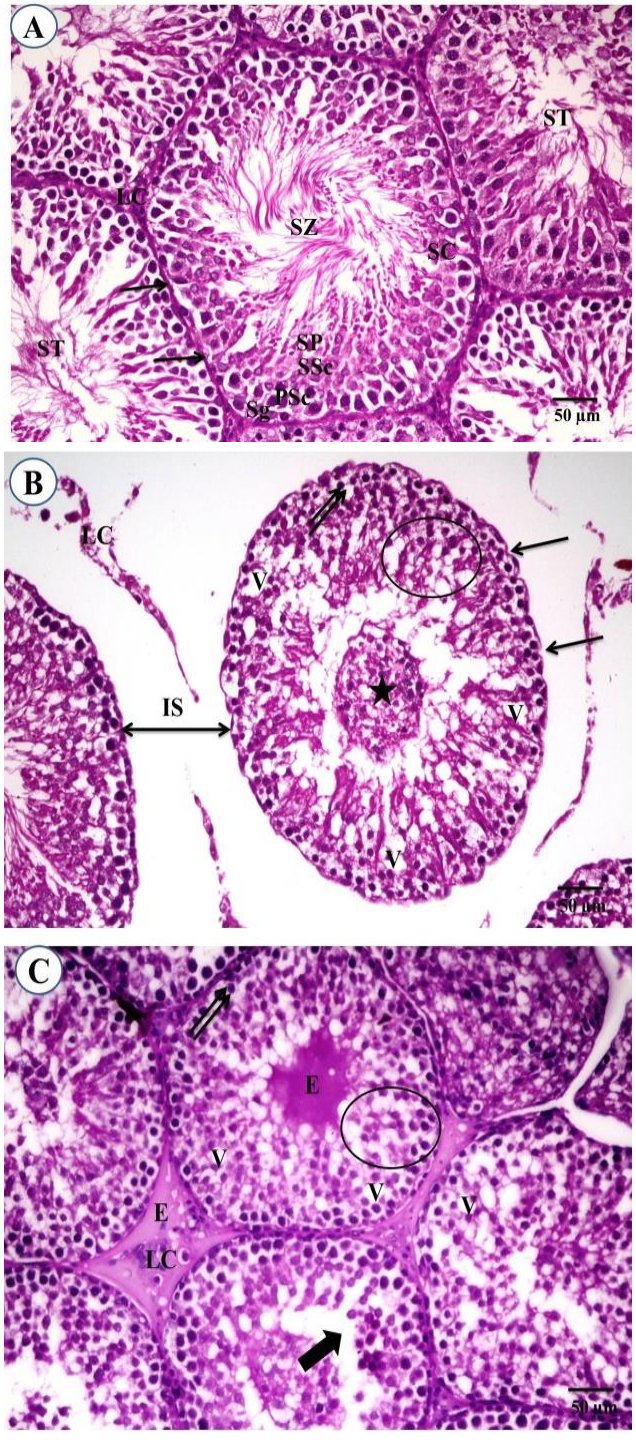


**Fig. 4 (A-C).** H&E-stained photomicrographs of testicular sections from different experimental groups. A The testes of the control rat exhibits many seminiferous tubules (ST) that are surrounded by a thin, regular basement membrane (arrow). Each seminiferous tubule (ST) is lined with stratified germinal epithelium, including Spermatogonia (Sg), Primary spermatocytes (PSc), Secondary spermatocytes (SSc), and spermatids (SP) with intraluminal spermatozoa (SZ). Leydig cells (LC) are found in the thin interstitium that separates seminiferous tubules (ST). B CTX-injected group (i.p.) (GP2) showed severe damage which has deformed basement membranes (arrow) and severe distorted seminiferous tubules with marked reduction in germinal epithelium thickness (black circle). These tubules contain cells that are highly degenerated, having vacuolated cytoplasm (V) and pyknotic nuclei (double arrow). The lumen of seminiferous tubules displays sloughed germinal cells (black stars) and is devoid of spermatozoa. The interstitial space (IS) is wide and contains degenerated Leydig cells (LC). C seminiferous tubules in the CTX-administered group (P.O.) (Gp3) exhibit a considerable decrease in the thickness of the germinal epithelium (black circle). These tubules include extremely degenerated cells with pyknotic nuclei (double arrow) and vacuolated cytoplasm (V) wide spaces (thick arrow) between germ cells. Seminiferous tubules display edema (E) in the lumen and lack spermatozoa, while edema (E) and vacuolated Leydig cells (LC) can be observed in interstitial space (IS). (H&E, X400).

## 4. Discussion

This study demonstrated the physiological and histological differences between P.O. and i.p. CTX treatment in terms of testicular toxicity in rats. Since antioxidant indicators (SOD, CAT, and GSH) significantly decreased and MDA increased, especially in the i.p. injected group, these data demonstrated the oxidative stress caused by CTX in testicular tissue. These results

align with an earlier study that showed oxidative pathways mediate CTX's gonadotoxic effects. The testes of rats given CTX also showed significant decrease in SOD, CAT, activities and GSH level, suggesting compromised antioxidant defense, according to Abarikwu et al. (2012). Their study attributed this to the generation of ROS during hepatic metabolism of CTX, which causes oxidative injury in peripheral tissues, including the testes. Kanter et al. (2010) also observed significantly increased MDA levels and degeneration of seminiferous tubules in CTX-treated animals. The current data align with findings by Naghdi et al. (2016), who demonstrated that CTX administration led to disrupted redox balance, suppressed testosterone levels, and testicular structural damage. The oxidative stress markers in their study followed a similar trend, with more pronounced effects noted with higher doses or more direct exposure routes like i.p. injection. These variations are probably caused by pharmacokinetic considerations, such as avoiding first-pass metabolism and allowing for faster tissue exposure through i.p. The importance of oxidative stress in CTX-induced testicular injury is highlighted by this uniformity across investigations. It also highlights the significance of the route of delivery in assessing the degree of toxicity, which has significant ramifications for experimental designs and clinical application.

Male rats' serum levels of testosterone, LH, and FSH were shown to be considerably lowered by both i.p. and P.O. administration of CTX. Notably, the i.p. route caused more profound suppression of hormonal levels compared to the P.O route, suggesting a dose-dependent or bioavailability-related effect of CTX. These findings align with previous research indicating that levels of testosterone, LH, and FSH were decreased in the presence of CTX (Yahya et al., 2022). For instance, CTX has been shown to impair Leydig cell function, thereby reducing testosterone production (Chavaengkiat et al., 2024). The observed decline in LH and FSH levels might reflect a disrupted hypothalamic-pituitary-gonadal (HPG) axis, a mechanism supported by previous research indicating that CTX interferes with gonadotropin secretion (Levi et al., 2022). Thus, the reduction in circulating testosterone is supposed to be resulting from a direct poisonous effect of CTX on the Leydig cells. Under normal conditions, the production of GnRH from the hypothalamus stimulates the anterior pituitary gland to create FSH and LH, which subsequently induce the secretion of gonadal hormones (testosterone and estrogen) in the testes.

Decreased hypothalamic responsiveness to the negative feedback of androgens elevates GnRH secretion, resulting in increased gonadotropin and androgen production, which stimulates testicular expansion and the emergence of additional secondary sexual characteristics. Chemotherapy alters the pituitary gonadal axis by decreasing spermatogenesis in the testis and seminiferous tubules, which have gonadotropin receptors. The chemotherapeutic drug inhibits meiosis, making spermatogenesis impossible to detect that responsible for regulating FSH in treated rat. FSH and LH levels are lowered in this study, affecting spermatogenesis negatively due to chemotherapy (Shetty et al., 2006).

Steroidogenesis in male rats is promoted by hypothalamic gonadotropin-releasing hormone (GnRH), which induces the generation and release of LH, then it attaches to receptors on the membrane of Leydig cells, enhancing testosterone production. The decrease in LH levels may be attributable to impairment in the negative feedback regulation of the hypothalamic-pituitary axis. Furthermore, the malfunction of the pituitary in LH release is likely attributable to impairment of the cell membrane-mediated signaling systems responsible for LH secretion into the bloodstream (Yahya et al., 2022). Additionally, pharmacokinetic studies indicating that i.p. treatment leads to larger peak plasma concentrations and faster systemic absorption is consistent with the distinct hormonal suppression between P.O. and i.p. routes (Ali et al., 2014). The results also showed new evidence to the use of hormone levels as sensitive indicators in chemotherapy settings to evaluate testicular damage. (Sheet, 2024) pointed out the risk of infertility and gonadal toxicity during CTX treatment which may be permanent even after termination of the treatment. All things considered, this investigation supports the

knowledge that CTX, especially when administered systemically, offers a serious risk to the endocrine function of male reproduction. These findings emphasize the necessity of preventative measures to maintain fertility in patients receiving chemotherapy and the significance of taking route-dependent toxicity into account in clinical and experimental contexts.

According to the current investigation, CTX considerably increased the levels of TNF-α and IL-6 when compared to control groups, especially when injected i.p. These findings support and extend a previous study that demonstrated CTX-induced reproductive toxicity through both hormonal disruption and inflammatory pathways. Increased TNF-α and IL-6 levels observed in this study mirror the inflammatory response described by Naghdi et al. (2016), who reported that CTX induces oxidative and inflammatory stress in reproductive tissues. Oxidative stress induces nuclear factor-κB to become activated in tissues treated with cyclophosamide, which leads to tissue damage by producing cytokines like TNF-α and IL-6 (Caglayan et al., 2018). The elevated cytokine levels following i.p. injection further support the conclusion that this route intensifies tissue inflammation. Gungor-Ordueri et al. (2021) also emphasized the role of CTX in activating pro-inflammatory cascades and disrupting testicular immune balance. The IL-6 and TNF-α increase in this study align with their findings, confirming that inflammation contributes significantly to testicular damage. These results reinforce the evidence that CTX compromises male reproductive function through combined steroidogenic and inflammatory disruptions. In line with previous studies, the i.p. route results in more severe effects, highlighting the need for careful consideration of drug delivery methods in both clinical and experimental contexts.

These findings showed that testicular tissue was severely damaged in both the CTX-i.p injected group and the CTX-P.O. administered group. This damage is probably caused by the liver converting CTX into acrolein, which has harmful effects and produces ROS in the body. The current results are supported by Sarman et al. (2023), who found that CTX therapy significantly changed the testes' histological architecture. Likewise, testicular membranes' high content of polyunsaturated fatty acids renders this tissue especially susceptible to oxidative stress, as shown by Ghosh et al. (2018). Furthermore, the pathophysiology of CTX-induced male reproductive damage is strongly impacted by oxidative stress. The large amount of ROS produced by CTX may cause spermatogenesis to malfunction or even sperm death. CTX disrupts spermatogenesis, reduces germ cells, and degenerates seminiferous tubules. Following CTX injection, histological investigations have revealed thickening of the basement membrane, vacuolization of Sertoli cells, and atrophy of the seminiferous epithelium (Wu et al., 2024). In conclusion, CTX treatment results in histopathological changes in testicular tissues and unfavorable effects on testicular function for both i.p. injection and P.O. delivery. In contrast, the i.p. route of CTX injection demonstrated a greater unfavorable effect than the P.O. administration.

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