

# Histological Study of the Effects of Cyclophosphamide on Kidney and Testis in Wistar Albino Rats

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## Abstract

**Background:** Cyclophosphamide [CPA] is a chemotherapeutic anticancer drug known for its cytotoxic effects on cancerous and normal cells. In its inactive form *in vitro*, the drug exerts its biological action primarily through metabolites, with phosphoramidate mustard being a key component. It exerts its effects by introducing alkyl groups into DNA, causing irreversible cross-linkages and inducing cell death during the G2 and S phases of the cell cycle. The aim is to determine changes in the cytoarchitectural structure of the kidney and testis with a single high dose vs divided low doses of cyclophosphamide. **Material and Methods:** Adult male Wistar albino rats [150-200g] were distributed into 3 groups [six per group] with free access to food and water. Group 1 received 150 mg/kg cyclophosphamide once intraperitoneally. Group 2 received 50 mg/kg cyclophosphamide once a week for three weeks; the control group received saline through the same route. All rats were weighed and euthanised on day 21, and their kidneys and testes were studied for histological changes. **Results:** The average body weight of albino rats decreased by 7.58% [ $p < 0.05$ ] in the group which was treated with a high single dose of CPA. It was reduced by 4.8% [ $p < 0.05$ ] in the group that was treated with low divided doses compared to the control group. Rats treated with a single high dose exhibited small, dark haemorrhagic areas in kidney on gross examination. Microscopically, single high-dose CPA treatment [150 mg/kg] induced significant histological changes: thinning of the capsule, subcapsular hemorrhages, disrupted cytoarchitecture, enlarged veins, congested tubules with degeneration and casts, and atrophied glomeruli. Whereas, the kidney treated with low divided doses [50 mg/kg] showed less disruption of cytoarchitecture, maintained capsule lining, less degeneration of glomeruli. The nuclei within the cytoplasm of tubules appeared rounded with prominent darkly stained nucleoli and maintained a cuboidal shape. Histological changes in testis presented as increased connective tissue and distortion of cells in the seminiferous tubules with a single high-dose. Low divided doses treated testes appeared normal, with no notable alterations in the testis capsule, with expected stages of sperm maturation seen in seminiferous tubules. **Conclusion:** Noteworthy morphological changes were seen in the kidney in albino rats exposed to cyclophosphamide (CPA) single high dose. In contrast, minor changes were noted in response to low dose. In the testes treated with single high dose and no notable changes were seen in the divided low doses. It can be suggested that the drug is primarily metabolised by the liver and kidneys and has a limited impact on reproductive organs like the testis.

**Keywords:** Cyclophosphamide (CPA), histopathology, nephrotoxicity, chemotherapy, testicular toxicity.

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## INTRODUCTION

Cyclophosphamide [CPA], is a synthetic anticancer medicine belonging to the alkylating agents (nitrogen mustard group). Synthesized in 1958 by Arnold & Bureaux and First approved for use in United States in 1959.<sup>[1]</sup> By adding alkyl groups to DNA, CPA produces irreversible cross-links and causes cell death during the G2 and S stages of the cell cycle.<sup>[2]</sup> It is widely used to treat a variety of cancers, such as lymphomas, leukemias, breast cancer, and ovarian cancer. At lesser dosages, it is also used to treat autoimmune conditions like rheumatoid arthritis and lupus.<sup>[2]</sup>

It is commonly used in organ transplant treatment regimens to prevent rejection and in autoimmune diseases like Nephrotic syndrome, since it also demonstrates immunosuppressive effects. Without the need for corticosteroid supplements, CPA preserves immune suppression and avoids cellular/humoral rejections.<sup>[3]</sup> As a pro-drug, CPA undergoes activation in the liver, primarily

excreted through urine. Increased exposure to CPA metabolites causes hepatotoxicity, which manifests as elevated bilirubin and aminotransferase levels and other signs of liver failure associated with sinusoidal obstruction syndrome. Also, it causes nephrotoxicity by damaging renal tubules.<sup>[4]</sup> Although CPA has a variety of harmful effects, and is highly effective at eliminating cancer cells and causes immunosuppression.<sup>[5]</sup> In adult albino mice, oral treatment of 10 mg/kg CPA for 30 days resulted in

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delayed hypersensitive reactions and body weight loss.<sup>[6]</sup> The liver damage caused by CPA is mostly dose-dependent.<sup>[7]</sup> It results from CPA's impaired metabolism, specifically that of its metabolite, acrolein.<sup>[8]</sup> To mitigate toxicity, the present research explores the efficacy of low-dose CPA. Chronic administration of divided low doses has shown positive outcomes in mice with cancer cells. Administration of medication in daily low-dose regimens instead of intermittent large dose, is proven to be effective against solid and hematologic tumours in mouse models. Low dose regime is observed to exhibit anti-angiogenic activity and enhance anti-tumour effects, particularly in advanced non-squamous cell lung carcinoma models with combined therapy.<sup>[9,10]</sup> However, several experimental human and animal studies with CPA have also shown adverse effects on a variety of organs, such as degeneration in the liver with sinusoidal obstruction syndrome, nephrotoxicity and reproductive toxicity, such as histological alteration of seminiferous tubules of testes and epididymis.<sup>[11-13]</sup> The effects of a single high dose concentration that is lethal to the cells were the primary focus of earlier research.<sup>[4-6,8,10]</sup> In the present study, the Wistar albino rats received two dose regimes: a single and a low divided dose. Histological changes in the kidney and testis of rats after administration of low and high doses of CPA were analysed.

**MATERIALS AND METHODS**

This study was conducted at the All India Institute of Medical Sciences (AIIMS), Rishikesh, following approval from the Institutional Ethics Committee (IEC No. 494/IEC/IM/2024). Three groups of six male Wistar albino rats, each weighing between 150 and 200 grams, were collected. The rats had unrestricted access to food and water in their housing. Before the experiment and before the animals were sacrificed, their body weights were noted. Rats in Experimental Group 1 received an intraperitoneal injection of CPA at a single, high

dose of 150 mg/kg body weight. CPA was given intraperitoneally to rats in Experimental Group 2 once a week for three weeks at a low dose of 50 mg/kg body weight. For three weeks, the same route was used to inject an equivalent volume of diluents [0.9% sterile saline solution] once a week into the Control Group 3. On the twenty-first day, all the animals were weighed and sacrificed by ether inhalation. The anterior thoracic wall was dissected. The rat heart was identified, and a catheter was inserted from the left ventricle to the aorta to perfuse the organs with buffered formalin (10%).

To observe the alterations in cytoarchitecture, cellularity, cell structure, connective tissue, collagen, 7-micron sections of the kidney and testis that had been dissected were prepared for paraffin embedding and stained with Haematoxylin-Eosin and Masson's Trichrome. The histological changes in the kidney's cortex and the testis' seminiferous tubules were examined under a microscope.

Statistical analysis– The body weight of rats in the control and experimental groups was recorded before and after sacrifice. Recorded data of the body weight were tabulated and statistical analysis was done by using the paired sample t-test using statistical package for the social sciences (SPSS) Software version 23.

**RESULTS**

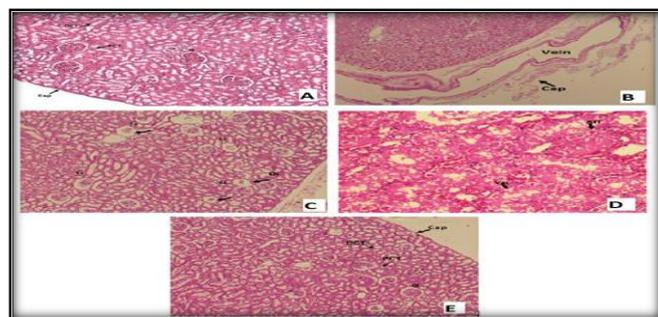
The mean body weight of animals was found to be significantly reduced [p < 0.05], by 7.6% in the single high dose and by 4.8% in divided low doses, as shown in [Table 1].

**Histological findings in kidneys**

**Haematoxylin & Eosin.** In the control group, the kidney exhibits typical histological architecture using the typical histological characteristics of the glomeruli, the cuboidal epithelial lining of the proximal convoluted tubules [PCT], and the distal convoluted tubules [DCT] are preserved, as illustrated in Figure 1-A under 40x magnification.

**Table 1: Shows comparisons of mean body weight of rats before experiment and before sacrifice in control and both experimental groups.**

Groups	Average weight before the experiment [Grams]	Average weight before sacrifice [Grams]	Percentage (%) change in body weight	P value [Paired t –test]
Control	185.00	189.17	2.2	0.224
Group 1 [High Dose]	175.83	162.50	7.6	0.005
Group 2 [Low Dose]	188.33	179.17	4.8	0.002



**Figure 1-A: Control group rat showing apparently normal renal cortex including capsule, proximal convoluted tubule [PCT] & distal convoluted tubules [DCT], rounded Glomeruli [G], [H&E 40x]. B: Cytoarchitecture beneath the capsule [Cap] was**

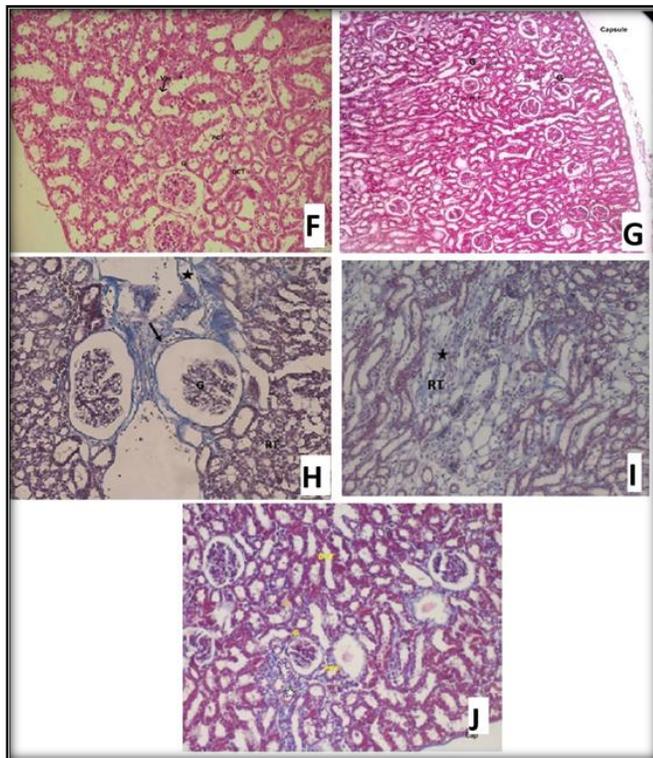
**completely disrupted and shows distended renal veins in [H&E] 10X. C: Glomeruli [G] were atrophied with widening [Di] (arrow marked) urinary [bowman's] space [H&E, 40x]. D: Kidney tissue shows vacuolated (Vac) cytoplasm and degeneration of tubular cuboidal epithelial cell [H&E, 100x]. E: Shows thinning of capsule [Cap] with few shrunken Glomeruli [G] and apparently normal PCT & DCT. [H&E, 40x].**

With high single dose of 150 mg/kg body weight CPA, on histology, the capsule showed distortion and disorganized cytoarchitecture beneath the capsule. The renal veins were enlarged and distended [Figure 1-B]. The glomeruli were atrophied with widening of the urinary [Bowman's] space [Figure 1-C]. The renal tubules show degeneration of tubular cuboidal epithelial cells with cytoplasm degeneration and ill-

defined vacuolated cytoplasm [Figure 1-D].

With low divided doses, the kidney showed thinning of the capsule. The cytoarchitecture deep into the capsule was less disrupted, with only a few shrunken, degenerated glomeruli [G]. The nuclei within the cytoplasm of tubules appeared rounded with prominent darkly stained nucleoli and apparently normal PCT and DCT [Figure 1-E]. Minimal cytoplasmic vacuolations [Vac] were seen in renal tubules [PCT] and [DCT] [Figure 2-F].

**Masson's trichome stain.** In control group, apparently normal collagen content in the form of fine collagen fibres between the tubules [PCT and DCT] was seen [Figure 2-G]. However, sections from kidneys treated with high dose CPA revealed peritubular and periglomerular fibrosis. Dense collagen fibers surrounded the glomeruli, glomerular capillaries and the renal tubules [Figures 2-H and 2-I].



**Figure 2-F:** Shows minimal vacuolations [Vac] in the tubules [H&E, 100X]. **G:** Represents normal collagen content as fine fibres between the tubules PCT & DCT in (40X, H&E). **H:** Masson trichome stain (MT) shows dense periglomerular collagen fibres (star marked), (arrowhead) & glomerulus (G). [40X, MT]. **I:** Shows (Star-marked area) dense collagen fibres among multiple tubules. [40X, MT]. **J:** Image shows less dense collagen fibers among the renal tubules [40X, MT].

Low- dose CPA treated kidney showed focal fibrosis of renal tubules. Changes demonstrated mild interstitial fibrosis, evidenced by less dense collagen fibres between renal tubules and around the glomeruli, as shown in [Figure 2-J]

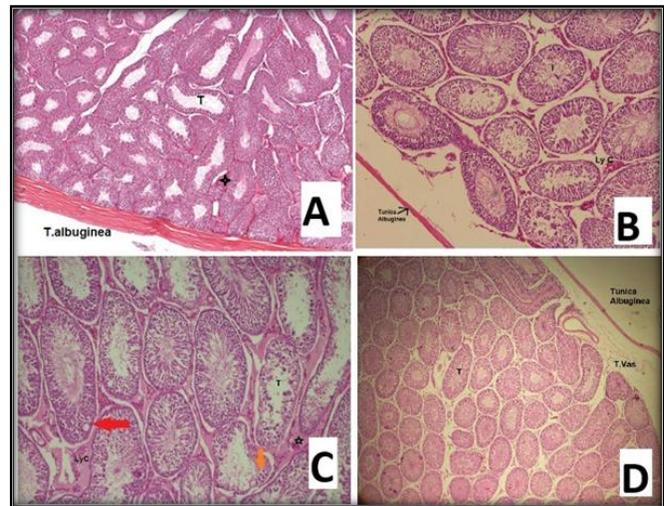
#### Histological findings in Testes

**Haematoxylin & Eosin.** Microscopic examination of the testes from control group revealed the normal histological features of the testis, including capsule (tunica albuginea),

and seminiferous tubules, Leydig cells, in addition to the interstitium between seminiferous tubules, as shown in [Figure 3-A].

Microscopic examination of the testis of rats given a single high dose of CPA showed thinning and separation of the tunica albuginea apart from the seminiferous tubule (T) as shown in [Figure 3-B]. Some seminiferous tubules (T) appeared distorted, with increased interstitial connective tissue (star), few spermatozoa cells, pyknotic nuclei (orange arrow), vacuolated cytoplasm (red arrow) and wide separation between the cells, [Figure 3-C].

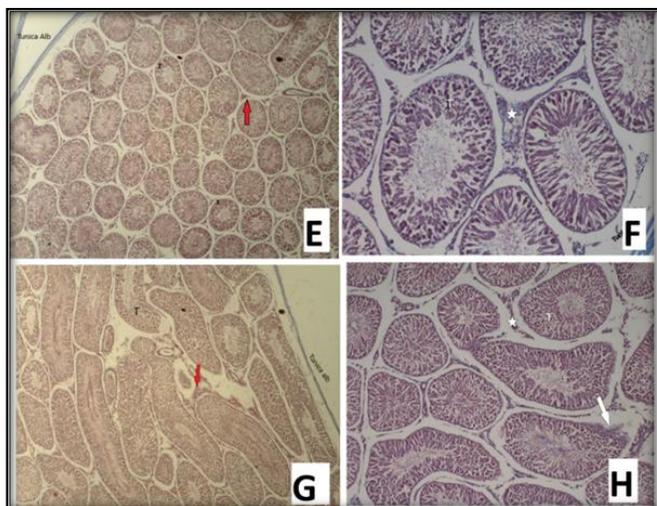
Microscopic examination of testis sections with H&E, of rats treated with a low dose of CPA, administered once a week for three weeks, revealed no significant changes. The seminiferous tubules showed no distortion or shrinkage of cells. Seemingly, normal interstitium is seen as shown in [Figure 3-D].



**Figure 3-A:** Shows normal histomorphological structure of testis in control group rats' such as leydig cell (Ly) & Seminiferous tubules(Sm). [H&E 100X]. **B:** Shows thinning & separation of tunica albuginea n between the seminiferous tubule (T), [H&E 40x]. **C:** Shows seminiferous tubules (T) are distorted, with increased interstitial connective tissue (star marked), shrunken mature spermatozoa cells in seminiferous tubules with extremely degenerated cells with pyknotic nuclei (orange arrow) and vacuolated cytoplasm (red arrow) with wide separation between the cells [H&E 100X]. **D:** Shows the normal outline tunica albuginea (Tunica alb), leydig cells present in the interstitium [Ly], rounded Seminiferous tubules [T] [H&E, 40X].

**Masson's Trichrome stain.** Control group with Masson's Trichrome stains shows no change in collagen content. Outer capsule (tunica albuginea) is intact. Interstitial spaces between tubules appear minimal, with no apparent fibrosis as shown in [Figure 4-E].

In single high dose CPA treated testis interstitial space shows mild expansion. The testicular architecture is mostly preserved, with mild degenerative changes likely indicative of early or low-level toxic or stress-related impact, as shown in [Figure 4-F].



**Figure 4-E:** Shows outer tunica albuginea (Tunica alb), seminiferous tubules (T) separated by normal interstitium (red arrow) [40X]. **F:** Shows seminiferous tubules (T) with relatively preserved architecture. Mild tubular dilation and slight disorganization of germinal epithelium are noted. Interstitial spaces (marked by star) are mildly expanded without prominent fibrosis or inflammatory infiltrate, suggesting early degenerative changes with partial maintenance of spermatogenesis. [100X, MT]. **G:** Shows outer intact tunica albuginea (Tunica alb), Seminiferous Tubules (T) are mostly round to oval with preserved overall architecture (marked by red arrow) [100X, MT]. **H:** Masson trichrome stain (MT) shows seminiferous tubules (T) with relatively preserved architecture. Mild tubular dilation and partial disorganisation of germinal epithelium are observed (marked by white arrow), with slightly reduced density of germ cells in some tubules, indicating early degenerative changes. [100X, MT].

In divided low dose CPA treated testis represents thin tunica albuginea, with less collagen content. Interstitial space appears apparently normal with normal collagen content as shown in [Figure 4-G & H].

## DISCUSSION

Cytotoxic alkylating class of medications includes cyclophosphamide [CPA], an anticancer medication used to treat a variety of neoplastic conditions. Hepatic cytochrome P450 enzymes bioactivate it, resulting in the production of its two metabolites, acrolein and phosphoramidate mustard. Acrolein, a by-product metabolite of CPA, interacts with protein amino acids to alter their structure and function, creates reactive oxygen species [ROS], and hinders the tissue antioxidant defence system.<sup>[9]</sup> The current study's findings demonstrate that Wistar albino rats given both high and low dosages of CPA experienced notable histological changes in their kidneys and testes.

When compared to the controls, cyclophosphamide significantly reduced the mean weight of all experimental animals [ $p < 0.05$ ]. Rats' weight decreased by 7.6% when given a high dose and 4.8% when given a low dose. Similar findings were noted by Emmenegger et al<sup>[10]</sup> in mice given both standard and low dosages of cyclophosphamide.

Researchers claim that CPA has no negative effects on the

kidneys. This is because acrolein is more urotoxic than nephrotoxic.<sup>[11]</sup> Nonetheless, we observed pathological alterations in our study, including the loss of brush border and pyknotic cells, oedema of tubular cells, and infiltration of acute inflammatory cells in the cortex. Similar alterations were also proposed by Sakr and Messady<sup>[14]</sup> who noted glomerular tuft atrophy, vacuolations in the renal tubules' epithelial lining, dilatation and congestion of the renal blood vessels following injection with CPA. As indicated by the rise in oxidative markers in the renal tissues, these alterations were attributed to oxidative stress brought on by CPA. Abraham and Rabi,<sup>[15]</sup> observed a decrease in glomerular filtration rate in addition to tubular dysfunction, glomerular nephritis, interstitial oedema, and cortical tubular vacuolization with CPA in rats. Similarly, Estakhri et al<sup>[16]</sup> noted a marked rise in blood urea nitrogen and creatinine levels as well as a histopathologic alteration in renal tissue. According to Sakr and Abdel-Samei,<sup>[17]</sup> the administration of CPA caused several histological changes in the kidney, such as atrophy of the glomeruli, leucocytic infiltrations, renal tubule degeneration, and congestion of the renal blood vessels. Additionally, there was an increase in urea and creatinine. Certain herbs like Ginkgo biloba extract, fennel oil<sup>[18]</sup> are claimed to mitigate these negative effects. According to Al-Amoudi,<sup>[18]</sup> fennel oil treatment or administration in combination with CPA improved the immunohistochemical and histological changes and recovered the creatinine and urea levels. While testes treated with lower doses of CPA did not exhibit significant histological alterations, testes treated with greater doses showed increased interstitial connective tissue, distortion, and cell shrinkage in seminiferous tubules. Similar results were obtained by El Gharabawy et al<sup>[19]</sup> who found thickening of the outer layer [tunica albuginea], deformed and undersized seminiferous tubules with large gaps between them. The thickness of the lining in tubules was significantly decreased. This layer appeared to be isolated from a thicker, uneven substrate, and there were no sperm cells in the large empty areas. Elkerdasy<sup>[20]</sup> reported that CPA induced damage to the normal histological structure of the testicles, increased deposition of collagen fibres, and markedly reduced the percentage of PCNA-immune-positive germ cells, suggesting a decline in spermatogenesis. These changes are in accordance with our study results which shows histological changes of testis like increase interstitial connective tissue, shrunken & distorted cell in seminiferous tubules treated with high dose of CPA.

## CONCLUSION

Cyclophosphamide, a widely used chemotherapeutic agent, is recognized for its nephrotoxic and urotoxic effects. Our study demonstrated significant pathological alterations in kidney histology among albino rats exposed to Cyclophosphamide. Moreover, our findings suggest that the histomorphological damage to the kidneys is dose dependent. Higher doses cause more damage while same dose administered in divided quantity leads to lesser destruction in kidney. Similarly, in contrast, our investigation shows increased connective tissue and distortion of cells in the seminiferous tubules with high-dose contrary to low-dose treated testes which appeared normal, with mild focal alterations in the testis. Expected stages of sperm maturation seen

in seminiferous tubules did not reveal notable changes in testicular morphology or function following cyclophosphamide administration. Minimal changes in the testis across different doses suggest that the drug, primarily filtered through the liver and kidneys, has limited impact on the reproductive organs like testis. Knowledge of dose dependent toxicity may be utilised in calibrating the dosage of Cyclophosphamide in various drug regimens to have optimum therapeutic effect while keeping the toxicity to minimum.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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