International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 01-02-2026, Accepted: 04-03-2026, Published: 19-03-2026
Volume 11, Issue 1, 2026, Page No. 543-550

Toxicological effects of cyclophosphamide on the testes: A mini review

S S Khan, V T Dhurvey, A M Nagwanshi*, A A Sharma
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India
Corresponding Author: A M Nagwanshi

Abstract
Cancer is a life-threatening disease caused by genetic mutations leading to uncontrolled cell proliferation. While chemotherapy has improved survival, many anticancer drugs produce significant adverse effects, particularly on the male reproductive system. Treatment-related gonadotoxicity has therefore become a major concern among cancer survivors. Cyclophosphamide (CP) is a widely used alkylating agent for the treatment of various malignancies and autoimmune disorders. Despite its clinical efficacy, CP is well known for its detrimental effects on male fertility. Experimental and clinical studies report reduced sperm count, motility, and viability, increased sperm abnormalities, and impaired fertilizing capacity. Histopathological changes include degeneration of seminiferous tubules, germ cell loss, and disruption of Sertoli and Leydig cells, leading to impaired spermatogenesis and testosterone production. Although some recovery may occur depending on dose and exposure duration, the extent and pattern of reversibility remain unclear. The present review aims to systematically compile and critically analyze existing experimental and clinical evidence regarding cyclophosphamide-induced male reproductive toxicity, focusing on the underlying mechanisms, structural and functional alterations, dose- and time-dependent effects, and the potential for recovery.

Keywords: Cyclophosphamide, male reproductive toxicity, testicular damage, spermatogenesis, sperm abnormalities

Introduction

Cancer is a disorder that results from genetic or epigenetic alterations in the somatic cells and has abnormal cell growth which may be spread to other body parts. It was predicted by Global demographic characteristics that about 420 million new cases of cancer by 2025 annually, which means increasing cancer incidence in years (Saini *et al.,*2020) [38]. Globally about 9.6 million in 2018 deaths were estimated in cancer which represents the cancer is the second leading cause of deaths and about 1 in 6 deaths are due to cancer. The commonest cancers are prostate cancer (1.28 million), female breast cancer (2.09 million), colorectal cancer (1.1 million), stomach cancer (1.03 million) and non-melanoma skin malignancies (1.04 million). There are various types of cancer treatments, surgery, chemotherapy, immunotherapy and radiation therapy which depend upon the cancer type (Saini *et al.,*2020) [38].

Cyclophosphamide (CP; $C_7H_{15}Cl_2N_2O_2P$, with a relative molecular mass of 279.10 g⁄mol; N-bis(2-chloroethyl)1-oxo-6-oxa-2-aza-1k5-phosphacyclohexan-1-amine hydrate) (Fig. 1) belongs to the class of oxazaphosphorines is odourless, fine white crystalline powder that is soluble in ethanol and water (Supriya *et al.,* 2022) [42]. It is a cytotoxic alkylating agent, extensively used in chemotherapy as an antineoplastic agent for the treatment of various cancers, as well as an immunosuppressive agent for organ transplantation, systemic lupus erythematosus and other benign diseases (Selvakumar *et al.,* 2006) [41]. CP is commonly used in the treatment of various malignancies, including Hodgkin and non-Hodgkin lymphomas, lymphocytic lymphomas, small lymphocytic lymphomas, Burkitt's lymphomas, and multiple myelomas, especially at Ann Arbor Stage III and IV (Murata *et al.,* 2004) [31]. Additionally, it is used to treat ovarian adenocarcinoma, disseminated neuroblastoma, retinoblastoma, nephrotic syndrome, breast cancer, leukemia, and retinoblastoma. To prevent graft rejection and complications related to graft-versus-host disease, CP is also used as an immunosuppressant before transplantation (Murata *et al.,* 2004; Olowe *et al.,* 2024) [31, 32].

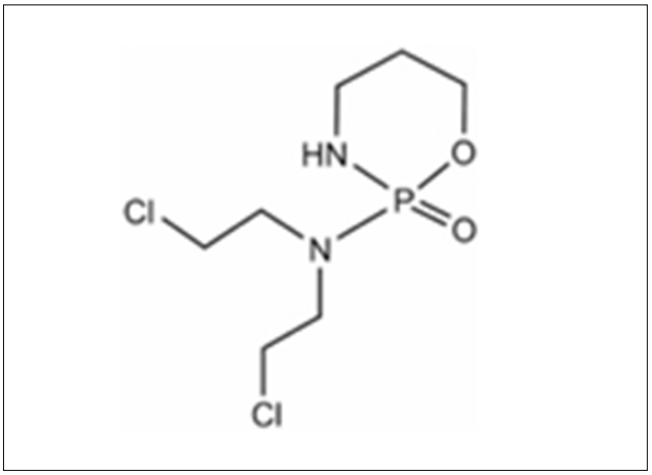

Fig 1: Chemical structure of cyclophosphamide. (Olowe *et al*., 2024) [32]

Mechanism of Action of Cyclophosphamide

Cyclophosphamide (CP) induces testicular toxicity primarily through two major mechanisms: DNA alkylation leading to germ cell apoptosis, and oxidative stress mediated by reactive oxygen species (ROS) (Fig. 2). The first mechanism involves the formation of DNA cross-links (Ghobadi *et al*., 2016) [18]. CP after metabolism produces two active metabolites, phosphoramide mustard and acrolein. These metabolites are bi-functional alkylating in nature that can potentially produce DNA-DNA or DNA-protein cross-links and induce single strand breaks by alkylating the DNA at N7 position of guanine (Tripathi and Jena 2008) [46]. Phosphoramide mustard exerts cytotoxic effects by forming DNA cross-links, thereby inhibiting

DNA replication and transcription, which ultimately leads to cell cycle arrest and apoptosis of rapidly dividing spermatogenic cells (Emadi *et al*., 2009; Ghobadi *et al*., 2017) [16, 18].

The second major mechanism is oxidative stress. The toxic metabolite acrolein promotes excessive generation of reactive oxygen species (ROS), resulting in lipid peroxidation, protein oxidation, and DNA fragmentation within testicular tissue (Ilbey *et al*., 2009; Selvakumar *et al*., 2006) [23, 41]. Increased oxidative stress disrupts the antioxidant defence system by reducing levels of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, thereby enhancing cellular damage in germ cells and Sertoli (Selvakumar *et al*., 2006) [41]. Cyclophosphamide also impairs endocrine regulation of spermatogenesis by affecting Leydig cell function and testosterone synthesis. Several studies report decreased serum testosterone levels following CP exposure, which is associated with degeneration of Leydig cells and altered hypothalamic–pituitary–gonadal axis signalling (Elangovan *et al*., 2006; Abarikwu *et al*., 2012) [1, 15].

Anticancer drugs, while effective in eliminating malignant cells, also exert cytotoxic effects on normal, healthy tissues. The testes, as the primary male reproductive organs, are responsible for the production of male gametes (spermatozoa) and the synthesis of testosterone, the principal male sex hormone that regulates secondary sexual characteristics and reproductive. function. Any structural damage or functional impairment of the testes can lead to decreased sperm count and reduced testosterone levels, ultimately resulting in male infertility. Therefore, the present review aims to evaluate the impact of cyclophosphamide (CP) on the histological architecture and biochemical parameters of the testes.

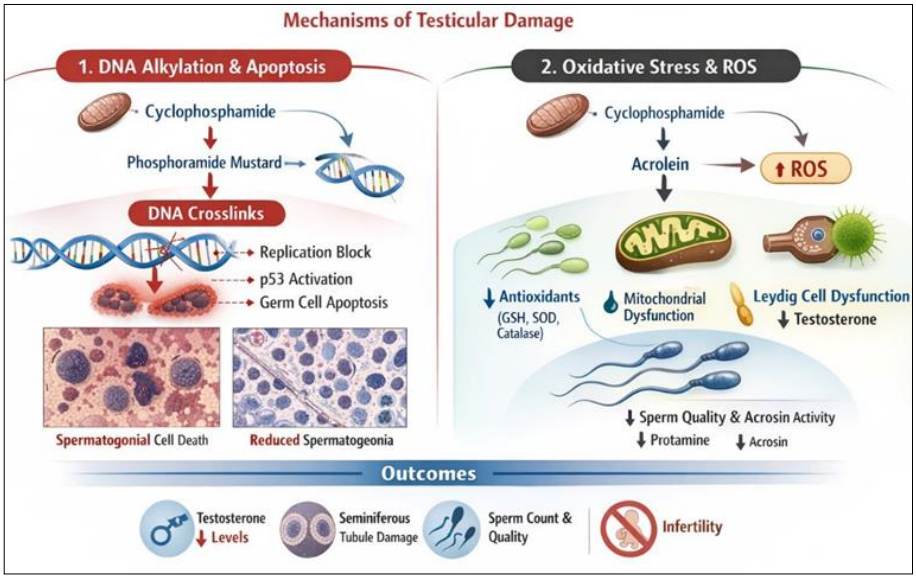

Fig 2: Mechanism of Cyclophosphamide induced testicular toxicity

Methodology

The authors followed PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Recommendations are a minimal collection of things based on evidences. The author focused on both experimental and non-experimental studies, using four internet databases including Google Scholar, PubMed, and Science Direct. Research articles published between and were searched for using these search engines. Research articles published between 2000 and 2025 were searched for using search engines.

Effects cyclophosphamide on testicular toxicity

The testes (testicles) are the primary male reproductive glands (gonads) responsible for producing sperm (spermatogenesis) and testosterone, which regulates male development and sexual function. The testes are composed of three principal cell types: spermatogonia or germ cells that develop into mature sperm, Sertoli cells that support and nurture developing germ cells, and Leydig cells that are responsible for testosterone synthesis (Tiwana and Leslie, 2023) [44]. Testes are highly susceptible to toxicant-induced damage, which often results in reduced sperm count, impaired fertility, and disrupted spermatogenesis. Multiple experimental studies have consistently demonstrated significant reductions in serum testosterone levels following CPA exposure (Elangovan *et al*., 2006; Ghosh *et al*., 2002; Samak *et al*., 2025) [15, 19, 40]. The germ cells are particularly susceptible to injury by cytotoxic drugs (Hobbie *et al*.,2004) [20].

1. Effect of Cyclophosphamide on Body Weight and Testis Weight

Body weight serves as an indicator of overall health, while testicular weight and dimensions are particularly crucial in toxicity studies (Benbella *et al*., 2025) [6]. Several experimental studies report significant reductions in both body weight and relative testicular weight following cyclophosphamide administration (Elangovan *et al*., 2006; Ilbey *et al*., 2009; Salimnejad *et al*., 2018) [15, 23, 39]. The decrease in body weight is associated to systemic toxicity, reduced food intake, metabolic stress, and chemotherapeutic exposure. Testicular weight reduction is primarily associated with seminiferous tubular atrophy, germ cell depletion, interstitial oedema, and Leydig cell degeneration. Loss of spermatogenic cells and shrinkage of seminiferous tubules contribute to reduced gonadal mass. (Ceribasi *et al*., 2010; El Gharabawy *et al*., 2019) [9, 14].

2. Histopathological Changes Induced by Cyclophosphamide

Histopathological examination of testicular tissue following cyclophosphamide exposure consistently reveals severe structural damage (Anan *et al*., 2017; El Gharabawy *et al*., 2019) [5, 14]. The antineoplastic effects of cyclophosphamide are associated with phosphoramide mustard, whereas acrolein is linked to toxic side effects like cell death, apoptosis, oncosis and necrosis (Jalali *et al*., 2011) [24]. Cyclophosphamide induced testicular toxicity is one of the most frequently reported adverse effects in males exposed to chemotherapy. Numerous experimental and clinical studies demonstrate that CPA causes severe impairment of spermatogenesis, leading to reduced sperm count, motility, viability, and increased abnormal sperm morphology (Mahecha *et al*., 2001; Selvakumar *et al*., 2006) [3, 41]. Common findings include degeneration and atrophy of seminiferous tubules, thinning of the germinal epithelium, widening of tubular lumens, and loss or exfoliation of germ cells into the lumen (Anan *et al*., 2017; Ceribasi *et al*., 2010; Elangovan *et al*., 2006) [5, 9, 15].

Several studies reported vacuolization and degeneration of Sertoli cells, formation of multinucleated giant cells, and disruption of the basement membrane, indicating impaired support for germ cell development (Bieber *et al*., 2006; Ilbey *et al*., 2009; Wtwt *et al*., 2012) [7, 23, 29, 48]. Leydig cells also exhibit ultrastructural alterations such as swollen mitochondria, dilated endoplasmic reticulum, and reduced cellular density, contributing to impaired steroidogenesis (Anan *et al*., 2017; El Gharabawy *et al*., 2019) [5, 14]. Dose-dependent severity of histological damage has been observed, with higher doses causing incomplete or arrested spermatogenesis and minimal recovery even after drug withdrawal (Drumond *et al*., 2011; Supriya *et al*., 2022) [13, 42].

Table 1: Histological changes in testes induced by Cyclophosphamide

Model organism	Dose and Duration	Histological Changes	References
Rat *(Rattus norvegicus)*	70 mg/kg body weight for 16 hrs	Degeneration of seminiferous tubules; loss of germ cells	Mahecha *et al.,* (2001)
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Reduced germ cells and spermatogenesis decreased	Ghosh *et al.,* (2002) [19]
Rat *(Rattus norvegicus)*	15 mg/kg for 10 weeks	Sperm abnormalities	Selvakumar *et al.*, (2005) [41]
Mice *(Mus musculus)*	50–200mg/kg body weight for 5 weeks	Reduced testis & epididymis size, tubular damage	Elangovan *et al.,* (2006) [15]
Rat *(Rattus norvegicus)*	Sub-chronic: 100 mg/kg (Day 1) + 50 mg/kg (Days 2–4); Chronic: 6 mg/kg/day	Germ-cell phase-specific sensitivity; mid-spermiogenic spermatids most affected	Codrington *et al.,* (2007) [11]
Rat *(Rattus norvegicus)*	6 mg/kg/day for 28 days	Impaired spermatogenesis and fertility	Rezvanfar *et al.,* (2008) [37]
Mice *(Mus musculus)*	50–200 mg/kg/week for 5 weeks	↓ Epididymis and testis weight, germ cell apoptosis, testicular degeneration	Tripathi and Jena (2008) [46]
Mice *(Mus musculus)*	100–250 mg/kg for 6 weeks	Reduced testicular weight; abnormal spermatozoa	Kanno *et al.,* (2009)
Rat *(Rattus norvegicus)*	100 mg/kg for 5 days	Irregular seminiferous tubules; germ cell loss	Ilbey *et al.,* (2009) [23]
Rat *(Rattus norvegicus)*	15 mg/kg/week for 8 weeks	Necrosis & vacuolation of seminiferous tubules	Ceribasi *et al.,* (2010) [9]
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Decreased body, testis & epididymis weight, degeneration and loss of germ cells, reduced seminiferous tubules	Jalali *et al.,* (2011)
Mice *(Mus musculus)*	150–200 mg/kg multiple doses every 4 days for 24 days	Depletion of differentiating germ cells, partial recovery of spermatogenesis	Drumond *et al.,* (2011) [13]
Rat *(Rattus norvegicus)*	15 mg/kg twice weekly for 4 weeks	Reduced sperm production and abnormal sperm morphology	Abarikwu *et al.,* (2012) [1]
Rat *(Rattus norvegicus)*	200 mg/kg single dose	↓ Johnsen score; disrupted germ cell maturation; interstitial degeneration	Ramos *et al.,* (2013)
Rat *(Rattus norvegicus)*	150 mg/kg single dose	Degeneration of spermatogonia and reduced spermatids/spermatocytes, ↓Sperm head count; ↓sperm motility	Kim *et al.,* (2013) [27]
Rat *(Rattus norvegicus)*	100 mg/kg for 6 weeks	Degeneration, vacuolation, exfoliation of germ cells; ↓ seminiferous epithelium thickness	Mohammadi *et al.,* (2013)
Rat *(Rattus norvegicus)*	7.5 mg/kg on 12, 13 and 14 day of gestation	Reduced testis weight, smaller seminiferous tubules; ↓ germ cells; tumor formation; persistent infertility	Comish *et al.,* (2014) [12]
Rat *(Rattus norvegicus)*	15 mg/kg/week for 5 weeks	Tubular atrophy, reduced spermatogenic cells	Suryaraj *et al.,* (2016) [43]
Rat *(Rattus norvegicus)*	15 mg/kg twice weekly for 4 weeks	Impaired sperm count, motility, daily sperm production, and increased abnormal sperm morphology	Ghobadi *et al*., (2016) [18]
Mice *(Mus musculus)*	150 mg/kg/day for 2 days	↓ Testicular weight, ↓ sperm count & motility, degeneration of spermatogonia & spermatocytes; structural testicular damage	Onaolapo *et al.,* (2017) [33]
Rat *(Rattus norvegicus)*	60 mg/kg/week	↓ Body, testis & epididymis weight; ↓ sperm count, motility & viability; ↑ abnormal sperm morphology; seminiferous tubule degeneration	Torabi *et al.,* (2017) [45]
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Shrunken tubules, reduced epithelial thickness, wide lumen	Anan *et al.,* (2017) [5]
Mice *(Mus musculus)*	100 mg/kg/week for 5 weeks	Reduced epithelial thickness, cell detachment	Salimnejad *et al.,* (2018)

			[39]
Mice *(Mus musculus)*	100 mg/kg single dose	↓ Sperm count & quality; seminiferous tubule shrinkage & germ cell loss	Pavin *et al.,* (2018) [35]
Rat *(Rattus norvegicus)*	6.1 mg/kg/day for 50 days	↓ Sperm count & germ cells; severe seminiferous tubule damage	Hosseini *et al.,* (2018) [21]
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Thickened tunica albuginea, shrunken seminiferous tubules and widened interstitium with empty lumina	Gharabawy *et al.,* (2019)
Rat *(Rattus norvegicus)*	50 mg/kg (single or double dose)	Reduced testes weight, spermatocyte & spermatogonia apoptosis, empty seminiferous tubules	Al-Salih *et al.,* (2020) [4]
Mice *(Mus musculus)*	10 mg/kg/day for 5 days	Increased germ cell apoptosis	Liu *et al.,* (2020)
Mice *(Mus musculus)*	50. 100, 200, 250 mg/kg	Incomplete spermatogenesis, partial recovery at lower doses and minimal Leydig cell damage	Supriya *et al.,* (2022) [42]
Mice *(Mus musculus)*	150 mg or 250 mg	Reduced body & organ weights with decreased sperm count	Yser (2023) [50]
Mice *(Mus musculus)*	200 mg/kg for 5 days	Reduced body, testes & epididymis weight with decreased sperm motility, high abnormal sperm and Sertoli cell vacuolation	Kim *et al.,* (2023) [26]
Rat *(Rattus norvegicus)*	150 mg/kg for one week	Loss of sperm, disrupted cell layers, dead cells in lumen, swelling, and damaged Leydig and Sertoli cells and congested blood vessels	Ghanim (2024) [17]
Mice *(Mus musculus)*	100 mg/kg once a week	Reduced sperm density and quality, vacuolated cells, and decrease in the height of germinal epithelium cells with reduced seminiferous tubules and germ cells	Kordedeh *et al.,* (2024) [28]
Rat *(Rattus norvegicus)*	40 mg/kg for 28 days	Reduced testicular weight, sperm count, motility and loss of germinal epithelium, disorganized seminiferous tubules and congestion of blood vessels	Priya *et al.,* (2024)
Rat *(Rattus norvegicus)*	200 mg/kg for 8 days	Distorted tubules; Leydig cell degeneration	Samak *et al.,* (2025) [40]
Rat *(Rattus norvegicus)*	15 mg/kg/week for 8 weeks	Loss of germ cells, spermatogenesis halted	Cellat *et al.,* (2025) [8]

Biochemical Changes Induced by Cyclophosphamide

Biochemically, cyclophosphamide-induced testicular toxicity is strongly associated with oxidative stress. CP exposure significantly increases lipid peroxidation markers such as malondialdehyde (MDA) while simultaneously reducing antioxidant defences including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase, and glutathione-S-transferase in testicular tissue (Selvakumar *et al*., 2005; Oyagbemi *et al*., 2015) [41, 34]. Hormonal disturbances are also prominent, with consistent reductions in serum testosterone levels and alterations in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), reflecting Leydig cell dysfunction and disruption of the hypothalamic-pituitary-gonadal axis (Elangovan *et al*., 2006) [15]. Additionally, CP impairs steroidogenic enzymes such as 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase, further compromising androgen synthesis (Ghosh *et al*., 2002; Abarikwu *et al*., 2012) [1, 19]. Increased DNA damage, protein oxidation, and activation of apoptotic pathways in germ cells have also been widely reported, reinforcing oxidative stress as a key mechanism underlying CP-induced reproductive toxicity (Tripathi and Jena 2008) [46].

Table 2: Effects of Cyclophosphamide on Biochemical Parameters

Model organism	Dose and Duration	Biochemical Changes	References
Rat *(Rattus norvegicus)*	70 mg/kg body weight for 16 hrs	Increased stress response genes	Mahecha *et al.,* (2001)
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	↓ Testosterone, ↓ 3β-HSD & 17β-HSD, ↑ oxidative stress	Ghosh *et al.,* (2002) [19]
Rat *(Rattus norvegicus)*	15 mg/kg for 10 weeks	↑ Lipid peroxidation; ↓ sperm count & motility	Selvakumar *et al*., (2005) [41]
Mice *(Mus musculus)*	50–200mg/kg body weight for 5 weeks	↓ Testosterone, LH, FSH	Elangovan *et al.,* (2006) [15]
Rat *(Rattus norvegicus)*	6 mg/kg/day for 28 days	↑ LPO; ↓ Total antioxidant power; ↓ Testosterone	Rezvanfar *et al.,* (2008) [37]
Mice *(Mus musculus)*	50–200 mg/kg/week for 5 weeks	↑ ROS; ↑ DNA damage; ↓ antioxidants	Tripathi and Jena (2008) [46]
Rat *(Rattus norvegicus)*	100 mg/kg for 5 days	↑ MDA; ↓ antioxidant enzymes	Ilbey *et al.,* (2009) [23]
Rat *(Rattus norvegicus)*	15 mg/kg/week for 8 weeks	↑ MDA; ↓ CAT, SOD, GSH	Ceribasi *et al.,* (2010) [9]
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Increased oxidative stress, reduced antioxidant defence	Jalali *et al.,* (2011)
Rat *(Rattus norvegicus)*	15 mg/kg twice weekly for 4 weeks	↓ Antioxidants (SOD, CAT, GSH-Px, GR, GST); ↑ MDA & LDH; ↓ 3β-HSD & 17β-HSD	Abarikwu *et al.,* (2012) [1]
Rat *(Rattus norvegicus)*	100 mg/kg for 6 weeks	↓ Testosterone, antioxidant levels and hair loss	Mohammadi *et al.,* (2013)
Rat *(Rattus norvegicus)*	200 mg/kg for 14 days	↓ LH, FSH, Testosterone and ↑ MDA, nitrite	Oyagbemi *et al.,* (2015) [34]
Rat *(Rattus norvegicus)*	15 mg/kg twice weekly for 4 weeks	Decreased antioxidant enzymes and increased MDA levels, along with affecting steroidogenic	Ghobadi *et al*., (2016) [18]

		enzymes	
Mice *(Mus musculus)*	150 mg/kg/day for 2 days	↓ Testosterone, GSH, SOD, CAT; ↑ MDA	Onaolapo *et al.,* (2017) [33]
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Increased oxidative stress	Anan *et al.,* (2017) [5]
Mice*(Mus musculus)*	100 mg/kg/week for 5 weeks	↓ Antioxidants (SOD, CAT), ↑ MDA	Salimnejad *et al.,* (2018) [39]
Mice *(Mus musculus)*	100 mg/kg single dose	↑ Oxidative stress, lipid peroxidation, and ↓ antioxidant enzymes (SOD, CAT, GP, GST, and GR), ↓ testosterone levels	Pavin *et al.,* (2018) [35]
Rat *(Rattus norvegicus)*	6.1 mg/kg/day for 50 days	↓ Testosterone, ↑ MDA	Hosseini *et al.,* (2018) [21]
Rat *(Rattus norvegicus)*	5 mg/kg/day for 28 days	Oxidative imbalance	Gharabawy *et al.,* (2019)
Mice *(Mus musculus)*	10 mg/kg/day for 5 days	↓ Testosterone	Liu *et al.,* (2020)
Mice *(Mus musculus)*	150 mg or 250 mg	↑ LH, ↓ Testosterone	Yser (2023) [50]
Mice *(Mus musculus)*	200 mg/kg for 5 days	↓ GSH, GR, CAT, ↑ MDA	Kim *et al.,* (2023) [26]
Mice *(Mus musculus)*	100 mg/kg once a week	↓ Testosterone, ↑ MDA	Kordedeh *et al.,* (2024) [28]
Rat *(Rattus norvegicus)*	40 mg/kg for 28 days	↓ Testosterone, ↑ Oxidative stress	Priya *et al.,* (2024)
Rat *(Rattus norvegicus)*	200 mg/kg for 8 days	↓ Testosterone, LH, FSH; ↓ antioxidants	Samak *et al.,* (2025) [40]
Rat *(Rattus norvegicus)*	15 mg/kg/week for 8 weeks	↑ Inflammation, ↓ antioxidant enzymes	Cellat *et al.,* (2025) [8]

Ameliorative and Protective Strategies Against Cyclophosphamide-Induced Testicular Toxicity

Cyclophosphamide-induced testicular damage has prompted considerable interest in identifying protective strategies that can preserve male reproductive function during chemotherapy. Because oxidative stress is a major mechanism underlying CPA toxicity, most therapeutic approaches aim to strengthen the antioxidant defence system. Various ameliorative and protective strategies, primarily focused on antioxidant, anti-inflammatory, and anti-apoptotic agents, have been identified in preclinical studies (mostly in rats) to mitigate these effects (Abu-Risha *et al*., 2022) [2]. Natural compounds with antioxidant properties have shown significant potential in restoring testicular function, balancing oxidation-antioxidation levels, and protecting sperm parameters (Chen *et al*., 2025) [10].

Natural compounds such as rutin (Abarikwu *et al*., 2012; Ghobadi *et al*., 2016) [1, 18], Vitamin E (El Gharabawy *et al*., 2019) [14], melatonin (Ilbey *et al*., 2009) [23], gallic acid (Oyagbemi *et al*., 2015) [34], Tribulus terrestris (Pavin *et al*., 2018) [35], ginger extract (Mohammadi *et al*., 2014) [30], and astaxanthin (Tripathi and Jena 2008) [46] have shown protective effects in animal. Rutin acts as an antioxidant that protects germ cells from oxidative damage, while vitamin E preserves sperm membrane integrity by preventing lipid peroxidation. Melatonin enhances antioxidant defense and supports spermatogenesis, whereas gallic acid reduces oxidative stress and cell damage in testicular tissue. Tribulus terrestris may improve testosterone production and reproductive function. Ginger extract provides antioxidant and anti-inflammatory protection, and astaxanthin safeguards cellular membranes against oxidative injury. These agents reduce lipid peroxidation, restore antioxidant enzymes such as SOD and catalase, improve testosterone levels, and help maintain the structural integrity of seminiferous tubules.

Table 3: Ameliorative and Protective Effects Against Cyclophosphamide-Induced Testicular Toxicity

Model organism	Dose and Duration	Biochemical Changes	References
Rat *(Rattus norvegicus)*	CP – 5 mg/kg/day hCG - 5 I.U./kg/day for 28 days	hCG restored steroidogenic enzyme activity, ↑ testosterone, improved germ cell counts & spermatogenesis, ↓ oxidative stress	Ghosh *et al.,* (2002) [19]
Rat *(Rattus norvegicus)*	CP - 15 mg/kg orally and Lipoic Acid- 35 mg/kg i.p.	LA improved the semen quality and reduced the oxidative stress and DNA damage	Selvakumar *et al.*, (2005) [41]
Mice *(Mus musculus)*	CP- 50, 100, 200 mg/kg/week AST- 25 mg/kg for 5 weeks	AST treatment improved the testes weight, sperm count, reduced sperm abnormalities, minimized DNA damage.	Tripathi and Jena (2008) [46]
Rat *(Rattus norvegicus)*	CP- 6 mg/kg/day SKEO- 225 mg/kg/day with olive oil for 28 days	SKEO improved CP-induced changes increases TAP in plasma and testis, restored body weight, increased testosterone levels, sperm quality, spermatogenesis and fertility, toxic stress, and DNA damage.	Rezvanfar *et al.,* (2008) [37]
Rat *(Rattus norvegicus)*	CP- 100 mg/kg Melatonin- 10 mg/kg for 5 days	Melatonin restored antioxidant enzymes, ↓ oxidative stress, improved sperm quality & testosterone and preserved seminiferous tubule structure	Ilbey *et al.,* (2009) [23]
Rat*(Rattus norvegicus)*	CP- 15 mg/kg/week Lycopene- 10 mg/kg Ellagic acid- 2 mg/kg for 8 weeks	LC or EA treatments improved the CP-induced lipid peroxidation, sperm morphology and testicular histopathology.	Ceribasi *et al.,* (2010) [9]
Rat *(Rattus norvegicus)*	CP- 5 mg in 5 ml saline/kg/day *Crataegus monogyna*- 20 mg/kg/day for 28 days	*Crataegus* caused partial recovery of spermatogenesis, improved seminiferous tubule repopulation, improved organ weights	Jalali *et al.,* (2011)
Rat *(Rattus norvegicus)*	CP- 15 mg/kg twice weekly Rutin- 30 mg/kg in corn oil for 4 weeks	RUT restored antioxidants & steroidogenic enzymes, ↓ MDA & LDH and improved sperm parameters	Abarikwu *et al.,* (2012) [1]
Rat *(Rattus norvegicus)*	CP- 150 mg/kg single dose	DADS Improved sperm head count & motility	Kim *et al.,* (2013) [27]

	Diallyl disulfide (DADS)- 100 mg/kg for 3 days	and reduced histopathological damage in testis and epididymis	
Rat *(Rattus norvegicus)*	CP- 100 mg/kg single dose *Zingiber officinale* (300 & 600) mg/kg/day for 6 weeks	*Zingiber* ↑ Germ cell counts, restored epithelial thickness, ↑ antioxidants, ↑ testosterone and improved histology	Mohammadi *et al.,* (2013)
Rat *(Rattus norvegicus)*	CP- 200 mg/kg Gallic acid- 60, 120 mg/kg for 14 days	GA increased MDA, LH, FSH and testosterone, restored antioxidants and sperm quality	Oyagbemi *et al.,* (2015) [34]
Rat *(Rattus norvegicus)*	CP- 15 mg/kg twice weekly Rutin- 30 mg/kg for 4 weeks	Rutin prevented the decline in sperm quality, restored antioxidant and reproductive enzyme levels in both the testes and epididymis.	Ghobadi *et al*., (2016) [18]
Mice *(Mus musculus)*	CP- 150 mg/kg/day Maca- 500 and 1,000 mg/kg for 28 days	Maca improved sperm parameters, restored testosterone levels, reduced oxidative stress and preserved seminiferous tubule structure.	Onaolapo *et al.,* (2017) [33]
Rat *(Rattus norvegicus)*	CP- 60 mg/kg Melatonin- 10 mg/kg Zinc oxide- 5 mg/kg for 8 weeks	Combined effect of melatonin and nZnO was more effective, restoring sperm parameters, testicular weights and seminiferous tubule diameter.	Torabi *et al.,* (2017) [45]
Mice *(Mus musculus)*	CP- 100 mg/kg/week Ghrelin- 80 µg/kg/day for 5 weeks	Ghrelin reduced lipid peroxidation, ↑ antioxidant capacity, improved seminiferous tubule structure	Salimnejad *et al.,* (2018) [39]
Mice *(Mus musculus)*	CP- 100 mg/kg single dose *Tribulus terrestris*- 11 mg/kg for 14 days	TT restored antioxidant enzymes, ↑ testosterone, improved sperm count & quality and testicular structure	Pavin *et al.,* (2018) [35]
Rat *(Rattus norvegicus)*	CP- 6.1 mg/kg/day *American ginseng*- 500 mg/kg/day for 50 days	*American ginseng* restored testosterone, ↓ MDA & DNA damage, improved sperm count & seminiferous tubule structure	Hosseini *et al.,* (2018) [21]
Rat *(Rattus norvegicus)*	CP- 5 mg/kg/day Vit E- 200 mg/kg for 28 days	Vit E improved testicular structure, reduced tunica thickness, restored seminiferous structure	Gharabawy *et al.,* (2019)
Rat *(Rattus norvegicus)*	CP- 10 mg/kg 5-Fluorouracil- 10 mg/kg/day for 14 days	5-FU normalized lipid profile, ↓ cholesterol, triglycerides & LDL, restored HDL levels.	Yahya *et al.,* (2022) [49]
Mice *(Mus musculus)*	CP- 200 mg/kg China Dust- 20,40 mg/kg on 1, 3, 5 days	CD aggravated oxidative stress intensified CP-induced systemic and testicular toxicity	Kim *et al.,* (2023) [26]
Mice *(Mus musculus)*	CP- 100 mg/kg once a week *Silymarin*- 200 mg/kg alternate day for 35 days	*Silymarin* restored sperm density & quality, ↑ testosterone, ↑ antioxidant capacity, ↓ MDA, improved seminiferous structure	Kordedeh *et al.,* (2024) [28]
Rat *(Rattus norvegicus)*	CP- 40 mg/kg Hesperidin- 100 mg/kg/day for 28 days	Hesperidin improved sperm motility & density, restored testosterone, ↓ oxidative stress & inflammation, normalized seminiferous tubules	Priya *et al.,* (2024)
Rat *(Rattus norvegicus)*	CP- 15 mg/kg/week Safranal- 200 mg/kg/day for 8 weeks	Safranal restored sperm quality, increased testosterone & antioxidants, reduced inflammation, preserved testicular histology	Cellat *et al.,* (2025) [8]

Conclusion

The collective reviewed evidences clearly demonstrates that cyclophosphamide exerts pronounced toxic effects on the male reproductive system. Testicular damage following cyclophosphamide exposure is consistently characterized by disrupted spermatogenesis, degeneration of seminiferous tubules, loss of germ cells, hormonal imbalance, and compromised sperm quality. These effects are largely driven by oxidative stress, DNA damage, and apoptosis, with developing germ cells showing the highest sensitivity. Although the extent of damage varies with dose, duration, and route of administration, many studies report long-lasting or irreversible fertility impairment, particularly after high or repeated doses. Importantly, several experimental investigations indicate that antioxidants, natural compounds, and hormonal interventions can partially alleviate cyclophosphamide-induced reproductive toxicity, highlighting oxidative stress as a key therapeutic target. Overall, this review emphasizes the need for fertility-preserving strategies and protective adjunct therapies in patients undergoing cyclophosphamide treatment, especially in young males and long-term cancer survivors.

References

1. Abarikwu SO, Otuechere CA, Ekor M, Monwuba K, Osobu D. Rutin ameliorates cyclophosphamide-induced reproductive toxicity in male rats. Toxicology International,2012:19(2):207–214.
2. Abu-Risha SE, Mousa MA, Elsisi AE. Protective role of irbesartan against cyclophosphamide-induced testicular damage in rats via up-regulating PPAR-γ signaling and ameliorating NF-κB/NLRP3/IL-18 inflammatory axis. Life Sciences,2022:289:120218.
3. Aguilar-Mahecha A, Hales BF, Robaire B. Acute cyclophosphamide exposure has germ cell specific effects on the expression of stress response genes during rat spermatogenesis. Molecular Reproduction and Development: Incorporating Gamete Research,2001:60(3):302-311.
4. Al-Salih HA, Al-Sharafi NM, Al-Qabi SS, Al-Darwesh AA. The pathological features of cyclophosphamide-induced multi-organ toxicity in male Wistar rats. Systematic Reviews in Pharmacy,2020:11(6):45–49.
5. Anan HH, Andrusishina IN, Elkholy AM. Histological and immunohistochemical study of cyclophosphamide

effect on adult rat testis. International Journal of Science and Reports,2017:3(1):128-136.
6. Ait Benbella J, Housbane S, Kadil Y, Kabbali F, Ghicha I, Bazhar H, *et al*. Evaluation of the reversibility of cadmium-induced testicular toxicity following recovery alone or with zinc supplementation. International Journal of Environmental Research and Public Health,2025:22(3):454.
7. Bieber AM, Marcon L, Hales BF, Robaire B. Effects of chemotherapeutic agents for testicular cancer on the male rat reproductive system, spermatozoa, and fertility. Journal of Andrology,2006:27(2).
8. Cellat M, Yildiz Ş, Demir O. Safranal ameliorates cyclophosphamide-induced reproductive toxicity by enhancing antioxidant defense and restoring spermatogenesis in male rats. Andrology Research & Therapeutics,2025:9(2):88–99.
9. Ceribasi AO, Turk G, Sonmez M, Sakin F, Atessahin A. Toxic effect of cyclophosphamide on sperm morphology, testicular histology and blood oxidant-antioxidant balance, and protective roles of lycopene and ellagic acid. Basic & Clinical Pharmacology & Toxicology,2010:107(6):730-736.
10. Chen W, Qiu J, Hong Z, Yuan J, Wang Q, Zhou W. Protective role of gallic acid in sperm health and environmentally induced male infertility: A narrative review of human-relevant and translational preclinical studies. Reproductive Biology,2025:25(3):101055.
11. Codrington AM, Hales BF, Robaire B. Exposure of male rats to cyclophosphamide alters the chromatin structure and basic proteome in spermatozoa. Human Reproduction,2007:22(5):1431–1442.
12. Comish DA, Bi J, Guo J. In utero exposure to cyclophosphamide induces long-term reproductive toxicity in male and female rat offspring. Toxicology and Applied Pharmacology,2014:276(2):129–138.
13. Drumond AL, Weng CC, Wang G, Chiarini-Garcia H, Eras-Garcia L, Meistrich ML. Effects of multiple doses of cyclophosphamide on mouse testes: Accessing the germ cells lost, and the functional damage of stem cells. Reproductive Toxicology,2011:32(4):395–406.
14. El Gharabawy GS, Abd Allah EE, Amr IM, Elmitwalli M. Histological and immunohistochemical study of the effect of cyclophosphamide on testis of male adult albino rats and the possible protective role of vitamin E. The Egyptian Journal of Hospital Medicine,2019:77(6):5930–5946.
15. Elangovan N, Chiou TJ, Tzeng WF, Chu ST. Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. Toxicology,2006:222(1–2):60–70.
16. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: Golden anniversary. Nature Reviews Clinical Oncology,2009:6(11):638–647.
17. Ghanim AM. Histological alterations of testes and sexual glands during and after cyclophosphamide treatment in male albino rats. Journal of Experimental Histopathology,2024:15(1):45–56.
18. Ghobadi E, Moloudi K, Abaszadeh A, Babadi VY, Gholami M. Protective effects of rutin on cyclophosphamide-induced reproductive toxicity in male rats. Andrologia,2016:48(9):1249–1256.
19. Ghosh D, Das UB, Ghosh S, Mallick M. Testicular gametogenic and steroidogenic activities in cyclophosphamide-treated rats: Effect of hCG coadministration. Asian Journal of Andrology,2002:4(3):201–205.
20. Hobbie WL, Ginsberg JP, Ogle SK, Carlson CA, Meadows AT. Fertility in males treated for Hodgkin's disease with COPP/ABV hybrid. Pediatr Blood Cancer,2005:44:193–19.
21. Hosseini A, Mohammadi R, Gholami M, Malayeri AR. Protective effect of American ginseng on cyclophosphamide-induced testicular toxicity in rats. Andrologia,2018:50(7):e13019.
22. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: Damage and recovery. Journal of the National Cancer Institute Monographs,2001:(34):12–17.
23. Ilbey YO, Ozbek E, Simsek A, Otunctemur A, Cekmen M, Somay A. Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats. Fertility and Sterility,2009:92(3):1124-1132.
24. Jalali AS, Hasanzadeh S, Malekinejad H. *Crataegus monogyna* aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: Stereological evidences. Acta Medica Iranica,2012:50(1):1–8.
25. Kenney LB, Laufer MR, Grant FD, Grier H, Diller L. High risk of infertility and long-term gonadal damage in males treated with high dose cyclophosphamide for sarcoma during childhood. Cancer,2001:91(3):613-621.
26. Kim J, Lee H, Park S, Choi Y. Protective effects of China dust on cyclophosphamide-induced testicular damage in mice. Journal of Toxicological Pathology,2023:36(4):215–226.
27. Kim SH, Lee IC, Baek HS, Moon C, Kim SH, Kim JC. Protective effect of diallyl disulfide on cyclophosphamide-induced testicular toxicity in rats. Laboratory Animal Research,2013:29(4):204-211.
28. Kordedeh S, Rahimi F, Jorfi M. Protective role of silymarin against cyclophosphamide-induced testicular toxicity in mice. Andrology & Reproductive Biology,2024:8(2):112–120.
29. Bieber M, Marcon L, Hales BF, Robaire B. Effects of chemotherapeutic agents for testicular cancer on the male rat reproductive system, spermatozoa, and fertility. Journal of Andrology,2006:27(2):189–200.
30. Mohammadi F, Nikzad H, Taghizadeh M, Taherian A, Azami-Tameh A, Hosseini SM, *et al*. Protective effect of *Zingiber officinale* extract on rat testis after cyclophosphamide treatment. Andrologia,2014:46(6):680-686.
31. Murata M, Suzuki T, Midorikawa K, Oikawa S, Kawanishi S, Yamashita N. Oxidative DNA damage induced by 4-hydroperoxycyclophosphamide, an active form of cyclophosphamide, via hydrogen peroxide. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,2004:560(1):11–22.
32. Olowe OA, Adekunle AJ, Balogun FT. Cytotoxic properties of cyclophosphamide and its mechanistic impacts on male gonadal function. International Journal of Reproductive Biology,2023:12(1):33–42.
33. Onaolapo OJ, Onaolapo AY, Akanmu MA. *Lepidium meyenii* (Maca) attenuates cyclophosphamide-induced gonadotoxicity in male mice. Middle East Fertility Society Journal,2017:22(2):101–109.

34. Yagbemi AA, Omobowale TO, Azeez OI, Abiola JO, Adedokun RA, Nottidge HO. Gallic acid ameliorates cyclophosphamide-induced reproductive toxicity in male Wistar rats. Journal of Complementary and Integrative Medicine,2015:12(4):307–314.
35. Pavin NF, Izaguirry AP, Soares MB, Spiazzi CC, Mendez ASL, Leivas FG, *et al*. *Tribulus terrestris* protects the male reproductive system from cyclophosphamide-induced damage in mice. Oxidative Medicine and Cellular Longevity,2018:2018:5758191,1–9.
36. Amos S de P, Goessler KF, Ruiz RJ, Ferrari O, Polito MD, Salles MJS. Exercise protects rat testis from cyclophosphamide-induced damage. Acta Scientiarum. Biological Sciences,2012:35(1):105-113.
37. Rezvanfar MA, Sadrkhanlou RA, Ahmadi A, Shojaei-Sadee H, Rezvanfar MA, Mohammadirad A, *et al*. Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source: Evidence for role of free-radical toxic stress. Human & Experimental Toxicology,2008:27(12):901–910.
38. Saini A, Kumar M, Bhatt S, Saini V, Malik A. Cancer causes and treatments. International Journal of Pharmaceutical Sciences and Research,2020:11(7):3121–3134.
39. Salimnejad R, Soleimani Rad J, Mohammad Nejad D, Roshangar L. Effect of ghrelin on total antioxidant capacity, lipid peroxidation, sperm parameters and fertility in mice against oxidative damage caused by cyclophosphamide. Andrologia,2018:50(2):e12883.
40. Samak M, Al-Qahtani R, Hussain A. Comparative evaluation of intraperitoneal and oral cyclophosphamide exposure on testicular structure and hormonal balance in male rats. Toxicology Reports,2025:12:101–110.
41. Selvakumar E, Prahalathan C, Sudharsan PT, Varalakshmi P. Protective effect of lipoic acid on cyclophosphamide-induced testicular toxicity. Clinica Chimica Acta,2005:360(1–2):160–166.
42. Supriya S, Sharma D, Shukla R. Histological changes in the testis of mice following exposure to different doses of cyclophosphamide. International Journal of Anatomy and Research,2022:10(3):8420–8427.
43. Suryaraj G, Chidambaram J, Srinivasan S. Acute and sub-chronic effects of cyclophosphamide on sperm morphology and reproductive indices in male rats. International Journal of Pharmacy and Pharmaceutical Sciences,2016:8(10):109–113.
44. Tiwana MS, Leslie SW. Anatomy, Abdomen and Pelvis: Testes. In: StatPearls. StatPearls Publishing, Treasure Island (FL), 2025. PMID: 29261881.
45. Torabi F, Hesaraki S, Najafi G, Mohammadi M, Moradi A. Combined effects of zinc oxide nanoparticles and melatonin on cyclophosphamide-induced testicular and epididymal toxicity in rats. Andrologia,2017:49(10):e12755.
46. Tripathi DN, Jena GB. Astaxanthin inhibits cytotoxic and genotoxic effects of cyclophosphamide in mice germ cells. Toxicology,2008:248(2–3):96-103.
47. Vaisheva F, Delbes G, Hales BF, Robaire B. Effects of the chemotherapeutic agents for non-Hodgkin lymphoma, cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), on the male rat reproductive system and progeny outcome. Journal of Andrology,2007:28(4):578-587.
48. Wtwt MA, Farhood HF, Mostafa FJ, Jaber IA, Ali Al-Ameri QM, Gathwan KH, *et al*. The effect of cyclophosphamide on spermatogenesis in rats. Research in Pharmacy,2012:2(3):15-21.
49. Yahya RAM, Attia AM, El M Shkal K, Yehia MA, Azab AE. Antidyslipidemic effect of 5-fluorouracil against cyclophosphamide-induced dyslipidaemia in male albino rats. Journal of Clinical Research and Reports, 2022, 12(2).
50. Yser A. Influence of cyclophosphamide administration on reproductive development capability and fertility in male mice. Journal of Reproductive Toxicology Studies,2023:5(2):45–53.