Indian J. Applied & Pure Bio. Vol. 41(1), 557-564 (2026). **ISSN: 0970-2091**

Hepatotoxic effects of Cyclophosphamide : A Mini Review

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Abstract

Cyclophosphamide is among the most successful chemo-therapeutic drugs, still widely used over 50 years. It is a synthetic nitrogen mustard alkylating agent from the oxazaphosphorine group. It is used in treatment of various cancers (breast, ovarian, neuroblastoma, retinoblastoma) and autoimmune diseases, and is used as an immuno-suppressant in transplantation. Although cyclophosphamide (CP) is an effective anticancer drug, it has several adverse effects, including genital disorders, testicular damage, cardiotoxicity, nephrotoxicity, and hepatotoxicity. Therefore, the present review aims to provide a comprehensive analysis of the hepatotoxic effects of this widely used cyclophosphamide.

Key words : Cyclophosphamide, Anticancer drug, Hepatic Toxicity, Liver enzymes, Hepatocellular damage.

Cancer is a major global health problem responsible for one in six deaths worldwide. In 2020, about 19.3 million new cancer cases and 10 million deaths were recorded[10]. It remains a leading cause of mortality and a major barrier to increasing life expectancy, ranking first or second among causes of death before age 70 in most countries[26]. When normal cell division regulatory mechanisms fail or unregulated cell growth occurs, cancer develops. Every type of cancer has particular features and can develop in any part of the body[24]. Sometimes being referred to as a "genetic disease," cancer development includes environmental factors that interfere with cellular functions or cause mutations[18]. DNA mutations can also change healthy cells into precancerous and eventually malignant cells that can spread through the blood or lymph to create new tumors.

Cancers are grouped according to where they originate :

- Skin or internal organ linings can develop carcinomas.
- Sarcomas of connective tissue, muscle, cartilage, or bone
- Leukemias – blood and bone marrow
- Lymphomas – cancer of immune system cells.

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- Brain and spinal cord cancers (central nervous system)[7].

In India, cancer incidence was about 1 million cases annually in 2012 and is projected to reach 1.7 million by 2035. Larger number of patients are diagnosed at advanced stages, resulting in a high mortality rate due to limited healthcare access[15].

Cancer Treatment Approaches :

Traditional treatments include surgery, radiotherapy, and chemotherapy, either alone or in combination[10]. Surgery is most effective in early stages, while radiation and chemotherapy can damage healthy tissues. Chemotherapy began in the early 20th century, gaining momentum after World War II with the establishment of the Cancer Chemotherapy National Service Center in 1955 [11].

Cyclophosphamide :

One of the most efficient chemotherapic drugs is cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$), which is still in widespread use more than 50 years after it was created. It is a chemically produced mustard alkylating agent which is soluble in ethanol, water, and saline and is derived from the oxazaphosphorine compound group (Fig. 1)[21]. It developed in 1959 as a result of research on nitrogen mustards by Dr. Arnold Welch and Alfred Gilman[8]. The drug is used as an immune suppressant during transplantation and treats a variety of autoimmune disorders and cancers, including breast, ovarian, neuroblastoma, and retinoblastoma. The main cytotoxic metabolite of cyclophosphamide, phosphoramide mustard, is generated in the liver by the cytochrome P-450 enzymes. By alkylating DNA and producing cross-links at guanine N-7 positions, this substance prevents replication and induces apoptosis[21].

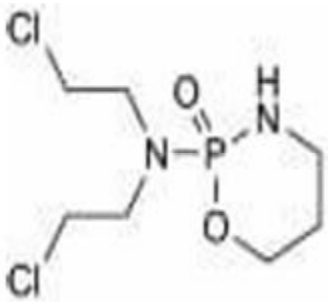

Figure 1. Structure of cyclophosphamide[21]

Despite being an effective anticancer drug, cyclophosphamide (CP) has a number of side effects, including hepatotoxicity, nephrotoxicity, cardiotoxicity, testicular damage, and genital disorders. Various histological and biochemical changes in tissues have been associated to CP's antineoplastic properties. Reactive oxygen species (ROS), which result in DNA fragmentation, necrosis, apoptosis, and cell death, are generated in excess when the equilibrium between free radicals and antioxidant defenses is disrupted[14]. Cyclophosphamide's urotoxicity, nephrotoxicity, and, to a lesser extent, hepatotoxicity are the main factors limiting its clinical use. The hepatotoxic effects of CP, however, are still up for debate. While some researchers believe the liver is comparatively resistant to the damage caused by CP, others suggest that it is highly susceptible to the cytotoxic effects of its metabolites[23]. Therefore, the present review aims to provide a comprehensive analysis of the hepatotoxic effects of this widely used anticancer drug, cyclophosphamide.

The author followed PRISMA, the Preferred Reporting ltems 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.

Histological changes :

Numerous research articles with histopathological analyses offer morphological indication of cyclophosphamide-induced hepatic damage. Hepatocyte degeneration, which represents cellular damage in liver cells which frequently manifests as swelling or altered morphology before progressing to necrosis along with cytoplasmic vacuolation, which is characterized by clear, empty-looking spaces in hepatocyte cytoplasm due to fluid accumulation or organelle swelling and frequently linked with metabolic stress[2]. Due to low lipid metabolism, progressive injury often leads to fatty change (steatosis)[13]. Vascular alterations consist of disruption of normal liver architecture, loosening of hepatocyte cords, and congestion of the central vein and sinusoids[19]. indicating enlarged, blood-filled liver sinusoids that block flow and are frequently caused by toxins or portal hypertension, resulting in hypoxia[25]. Central vein congestion damages upstream hepatocytes when blood backs up in the terminal hepatic venule, typically due to outflow obstruction. Inflammatory cell infiltration, in which lymphocytes, neutrophils, or macrophages invade the lobules or portals, frequently occurs along with these histological damages[1]. In severe cases, hepatocyte necrosis[20] results in nuclear alterations such as pyknosis, and bleeding causes red blood cell leakage into tissue.

According to (Montaser *et al*.,)[22] an increase in Kupffer cell proliferation indicates that the liver's resident macrophages have been activated in response to injury. Chronic cyclophosphamide exposure can lead to fibrosis[5], which is characterized by excessive connective tissue deposition; glycogen depletion, which is shown by decreased Periodic Acid–Schiff (PAS) staining[13], which shows impaired carbohydrate metabolism and reduced energy reserves in hepatocytes.

Effect on Histology :

Table-1. Comparative analysis of histological damage

Model organism	Dosage	Duration	Inference	Reference
Female Wistar rats *Rattus norvegicus*	150 mg/kg b.w.	Single day	Focal oedema and loosening of hepatocytes in the cords.	Abraham and Sugumar[2]
Albino rats *Rattus norvegicus*	0.5, 0.7 mg/ 100 g b.w.	12 weeks	Congestion of the central vein and sinusoids, loosening of hepatocyte cords, and disruption of normal liver architecture.	Khan *et al.,*[19]

NMRI mice *Mus musculus*	200 mg/kg b.w.	7 days	Necrosis, inflammation and hepatocytic infiltration.	Habibi *et al.,*[16]
Swiss Albino mice *Rattus norvegicus*	200 mg/kg b.w.	22 days	vacuolated hepatocytes that had either pyknotic or swollen nuclei. Pretreatment	El-Naggar *et al.,*[12]
Male albino rats *Rattus norvegicus*	150 mg/ kg b.w.	14 days	Congestion in the central vein and sinusoids associated with diffuse Kupffer cells proliferation.	Montaser *et al.,*[22]
Male mice *Mus musculus*	100 mg/ kg b.w	5 weeks	Hepatocyte necrosis, bleeding, inflammation, vacuolation, and sinusoidal dilation.	Khordad *et al.,*[20]
Female hamsters *Mesocricetus auratus*	0, 100, or 200 mg/ kg b.w.	1 week	Diffuse fatty infiltration, central vein, sinusoidal congestion and marked loss of hepatocyte architecture.	Elrashdy *et al.,*[13]
Mice *Mus musculus*	200 mg/ kg b.w.	7 days	Inflammatory infiltration, congestion, sinusoidal dilation, hemorrhage, and cytoplasmic eosinophilia.	Rezaei *et al.,*[25]
Male Wister rats *Rattus norvegicus*	50 mg/ kg b.w.	8 days	Fatty liver degeneration and inflammatory cell infiltration with additional mild amyloidosis and early fibrosis.	Al-Salih *et al.,*[5]
Male Albino rats *Rattus norvegicus*	5 mg/ kg b.w.	4 weeks	Vacuolation, infiltration, degeneration, and necrosis.	Abdul Hamid *et al.,*[1]
Male Albino rats *Rattus norvegicus*	150 mg/ kg b.w.	5 days	Damage in the portal tracts and central veins.	Amer *et al.,*[6] (2025)
Mice *Mus musculus*	300 mg/ kg b.w.	Single dose	Hepatocyte hydropic degeneration, necrosis, and inflammatory infiltration.	Wang *et al.,*[28]
Wistar Albino rats *Rattus norvegicus*	200 mg/ kg b.w.	4 days	Severe hepatic degeneration, inflammation, and necrosis.	Alam *et al.,*[4]

Biochemical changes :

It is also evident that cyclophosphamide administration can induces biochemical alterations, mainly hepatic dysfunction, oxidative stress, and disrupted metabolic homeostasis. Cyclophosphamide exposure caused significant elevation in serum liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), bilirubin, and hepatic MDA levels[3,4,12,27,28]. It also affects the serum protein profile causing significant reductions in total protein and albumin levels. Cyclophosphamide enhanced lipid peroxidation and oxidative damage to cellular membranes. Elevated MDA levels confirm excessive generation of reactive oxygen species and disruption of redox balance within hepatic tissue[4]. Additionally, cyclophosphamide causes a significant depletion of the hepatic antioxidant defense system. Both enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), and non-enzymatic antioxidants such as reduced glutathione (GSH) and total antioxidant capacity (TAC), are markedly reduced. This reflects in response to sustained oxidative stress[2,16].

Effect on Biochemical parameters :

Table-2. Comparative analysis of biochemicals changes

Model organism	Dosage	Duration	Inference	References
NMRI mice *Mus musculus*	200 mg/ kg b.w.	7 days	Increase in serum levels of hepatic markers	Habibi *et al.,*[16]
Swiss Albino mice *Rattus norvegicus*	200 mg/ kg b.w.	22 days	Elevation in serum liver enzymes	El-Naggar *et al.,*[12]
Sprague–Dawley rats *Rattus norvegicus*	200 mg/ kg b.w.	2 weeks	Alteration in enzymes	Ahmed[3]
Wistar Albino rats *Rattus norvegicus*	200 mg/ kg b.w.	4 days	Elevation in serum liver enzymes	Alam *et al.,*[4]
Wistar Albino rats *Rattus norvegicus*	200 mg/ kg b.w.	8 days	Elevated serum ALT, ALP, AST, and MDA levels,	Temel *et al.,*[27]
Female Wistar rats *Rattus norvegicus*	150 mg/ kg b.w.	Single day	Significant change in hepatic MDA levels was observed.	Abraham and Sugumar[2]
Male albino rats *Rattus norvegicus*	150 mg/ kg b.w	14 days	Increase in serum liver enzymes and decrease in total protein & albumin.	Montaser *et al.,*[22]

Male mice *Mus musculus*	200 mg/ kg, 80 mg/ kg b.w	14 days	Serum liver enzymes increased while reducing antioxidant enzymes activity.	Hao *et al.,*[17]
Mice *Mus musculus*	300 mg/ kg b.w.	Single dose	Elevation in plasma ALT and AST levels.	Wang *et al.,*[28]

Ameliorative effects :

Amelioration means improvement specifically, reducing the severity of a harmful effect and damage caused by a toxic agent. Cyclophosphamide-induced liver injury, ameliorative agents do not completely eliminate toxicity, but they help the liver cope with it and toxic effects becomes less severe. They often act by reducing oxidative stress, stabilizing cell membranes, suppressing inflammation, and strengthening the body's own antioxidant systems[9]. For instance, *Origanum vulgare* improved liver enzyme levels and restored hepatic structure in animals exposed to cyclophosphamide[16]. Likewise, vitamin E, Moringa leaf extract and saopinc acid limited lipid peroxidation, normalized antioxidant activity, and reduced microscopic liver damage[9,25]. Several experimental models support these observations; these studies show that ameliorative treatments play an important supportive role by reducing the biochemical and structural consequences of cyclophosphamide toxicity.

Table-3. Comparative analysis of Ameliorative effects

Model organism	Protective/ Ameliorative agent	Dose used (along with CP)	Ameliorative (protective) effects observed	Reference
NMRI mice *Mus musculus*	*Origanum vulgare*	50–400 mg/ kg b.w.	Showed ameliorative effect	Habibi *et al.,*[16]
Male Wistar rats *Rattus norvegicus*	Vitamin E	100 mg/kg b.w	Decreased MDA and apoptotic cells; improved hepatic structure	Cuce *et al.,*[9]
Swiss Albino mice *Rattus norvegicus*	Methanol extract of *Rosmarinus officinalis* leaves (MEROL)	100-200 mg/ kg b.w.	Showed protective effect	El-Naggar *et al.,*[12]
Male Albino rats *Rattus norvegicus*	Lipoic acid + Royal jelly	25 mg/kg, 1 g/kg b.w.	Showed ameliorative effect	Abdul Hamid *et al.,*[1]

Male albino rats *Rattus norvegicus*	Moringa leaf extract / Silymarin	500 mg/kg, 100 mg/kg b.w.	Showed ameliorative effect	Montaser *et al.,*[22]
Male mice *Mus musculus*	Ghrelin	80 µg/kg b.w.	Showed ameliorative effect	Khordad *et al.,*[20]
Mice *Mus musculus*	Saponic acid	5-10 mg/kg b.w.	Reversed ALT/AST rise and oxidative damage	Rezaei *et al.,*[25]
Male Albino rats *Rattus norvegicus*	Olive oil	200 mg/kg b.w.	Showed ameliorative effect	Amer *et al.,*[6]

From available literature review it is evident that Cyclophosphamide exhibits hepatic toxicity. Cyclophosphamide is an effective anticancer and immunosuppressive drug but causes significant dose-dependent hepatotoxicity. Severity of toxicity varies with species, dosage, duration, metabolic rate, and drug-administration schedule. Overall, combining cyclophosphamide therapy with suitable hepatoprotective agents may greatly improve patient safety and minimize liver toxicity. Further research and study is very important to understand cyclophosphamide's long-term health impacts and develop effective solutions for reducing its toxicity in liver.

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