



# Study of Leukopenia Induced by a High Dose of Cyclophosphamide in Mice

Idriss H. Mohamed\*

Zoology Department, Faculty of Science, Omar AL-Mukhtar University, Albida, Lybia

**ABSTRACT:** The study was conducted to evaluate the toxic effect of different concentrations of cyclophosphamide (CTX) by inducing leukopenia. Adult female Swiss albino mice weighting (20±4g) arranged in three groups of six animals each housed. A control mice saline PBS solution (group I), mice treated with 200 mg/Kg (4mg/mouse) cyclophosphamide, High dose (group II) and mice treated with treated 100 mg/kg (2mg/mouse) body weight Low dose (group III) for three weeks. Cyclophosphamide causes a significant decrease ( $P < 0.01$ ) in total leukocyte count (TLC), total erythrocyte count (TEC), and red blood cell distribution (RDW) in all groups compared to the control group of mice. Observed leukopenia inform marked reduced in the absolute numbers of lymphocytes, monocytes, and neutrophils in comparison to the control group of mice. The results indicate that cyclophosphamide alters the blood profile only after high doses while low doses had the least impact on blood pictures. Cyclophosphamide causes a significant increased ( $P < 0.01$ ) in activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum after CTX treatment with no effect on the serum albumin level compared to the control group. While lower doses had less effect on liver enzymes.

**Keywords:** Cyclophosphamide, cytotoxic effect, leukopenia, total erythrocyte count (TEC), Alanine amino transferase (ALT), total leukocyte count (TLC).

## RESEARCH PAPER

**\*Corresponding Author:**

*Idriss H. Mohamed*  
Zoology Department, Faculty of Science, Omar AL-Mukhtar University, Albida, Lybia

**How to cite this paper:**

Idriss H. Mohamed.; "Study of Leukopenia Induced by a High Dose of Cyclophosphamide in Mice". Middle East Res J Biological Sci, 2021 Nov-Dec 1(1): 9-13.

**Article History:**

| Submit: 24.10.2021 |  
| Accepted: 27.11.2021 |  
| Published: 28.12.2021 |

**Copyright © 2021 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

## INTRODUCTION

Cyclophosphamide (CTX) is a class of antineoplastic drugs, widely used in the treatment of various types of cancer [1]. Due to this cytotoxic property it is used extensively in a variety of carcinomas singly or in combination with other drugs to treat a variety of leukemias, Hodgkin's lymphoma, Multiple myeloma, Mycosis fungoides, lymphoma, breast cancer, ovarian cancer and Retinoblastoma. Cyclophosphamide is also used at lower doses for some autoimmune diseases such as systemic lupus erythematosus, severe rheumatoid arthritis, Wegener's granulomatosis, multiple sclerosis, some connective tissue disorders, minimal lesion glomerulonephritis, several forms of vasculitis, Nephrotic Syndrome and post organ transplantation for prevention of rejection of the organ [2]. Cyclophosphamide is a prodrug that gets activated to alkylating phosphoramidate mustard in the liver and excreted primarily (70%) in urine in forms of metabolites [3]. Adverse effects of cyclophosphamide include alopecia, thrombocytopenia, mucosal ulcerations, leukopenia, hematuria, diarrhoea, hemorrhagic cystitis and petechial haemorrhage in lungs and small bowel [4,5]. Cyclophosphamide is also

documented to cause hepatotoxicity in Fischer rats and cardiopulmonary toxicity in Mongrel dogs [6,7].

Recent studies suggest that CTX generates reactive oxygen species (ROS) like superoxide anion, hydroxyl radical, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) during its oxidative metabolism and depresses the antioxidant defense mechanisms in the liver [8-9]. Detailed analysis of this leucopenic effect in mice showed in our study, that establish the CTX decreasing effect on the absolute number of leukocytes in the peripheral blood (PBL) [10].

CTX is transformed via hepatic enzymes to active alkylating metabolites. CTX cytotoxicity is due to the formation of phosphoramidate mustard, which generates DNA crosslinks and leads to apoptosis [4]. Cells deficient in aldehyde dehydrogenase (ALDH), the enzyme involved in CTX detoxification, produce an active phosphoramidate metabolite. Low levels of ALDH are expressed in lymphocytes and, therefore, these cells are sensitive to CTX. Conversely, hematopoietic stem cells (HSCs) express high levels of ALDH and resist CTX-mediated cytotoxicity [1].

## MATERIAL AND METHODS

Adult female Swiss albino mice weighting (20±4g) were procured and arranged in three groups of six animals each housed with ad libitum access to food and water. Group I Control animals were given orally an equal volume of diluents (0.9% sterile saline solution) for three weeks. Group II for induction of leukopenia mice (n=6/group) were treated for three weeks with intraperitoneal (i.p.) injection of 200 mg/Kg (4mg/mouse) cyclophosphamide (High dose). Group III mice were treated intraperitoneal (i.p.) with cyclophosphamide in the dose of 100 mg/kg (2mg/mouse) body weight (Low dose) a for three weeks.

### Evaluation of Hematological Parameters:

Blood samples with anticoagulant EDTA were analyzed for hematological parameters of (TEC), (TLC) and (RDW) counts, and an absolute number of neutrophils, monocyte, and lymphocytes according to Feldman [11].

### Serum Biochemical Analysis:

The serum liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT) and the liver albumin were determined according to the manufacturer's instructions (Biosystem, Egypt) [12-13].

### Statistical analysis

Data are obtained from each experiment were analyzed by using Microsoft Excel (Seattle, WA). The differences between the experimental groups were assessed using the Student's t-test.  $P > 0.05$  was considered to indicate statistical significance by using Graph Pad Prism version 4.0 software (Graph Pad).

## RESULTS AND DISCUSSION

We found that mice received treatment with CTX induced sharp decrease in both in the total number of leukocytes as well as in the number of neutrophils. This treatment, however, induced decreases in the number of lymphocytes and monocytes. These data are consistent with data reported by [14] on the effect of CTX in mice.

Anaemia is a deficiency in the concentration of haemoglobin-containing red blood cells that is prevalent among cancer patients [15]. Leucopaenia is also a common adverse event in cancer therapy [16]. CTX treatment can reduce the number of TEC, TLC and RDW the peripheral blood analysis revealed the high-dose CTX treatments stably decreased the number of TLC and RBCs compared with that of the control group, which is consistent with the previous studies [17,18]. Meanwhile, our animal model showed a more sustained suppression on TLC and TEC. Unlike in previous reports, the number of TLC in the CTX treatment groups was increased, which needs further research.

With the findings of several workers. The decrease in the TEC, TLC concentration and RDW leading to anaemia and leucopenia in patients treated with cyclophosphamide was reported by [19]. The leucocytic depression (leucopenia) was also reported by [20] It may be concluded that by inhibiting haematopoiesis, cyclophosphamide has dropped TLC leading to leucopenia after prolonged treatment.

**Table 1: Effect of different treatments on the number of TEC, TLC, and RDW.**

Parameters	PBS	200mg/Kg (High dose)	100mg/kg (Low dose)
TLC (×10 <sup>3</sup> )	3.8±0.48	1.80±0.48**	2.00 ±0.41
TEC (×10 <sup>6</sup> )	5.98±0.16	1.71±0.30**	3.60±0.32
RDW (×10 <sup>4</sup> )	20.21±0.59	15.08±0.57*	17.65±3.17

CTX, cyclophosphamide; PBS, Phosphate buffer saline; **TLC**, total leukocyte count total erythrocyte count; **TEC**, total erythrocyte count; and **RDW**, Red blood cell distribution width; ns, non-significant; \*and\*\*significant at  $P \leq 0.05$  and 0.01, respectively.

**Table 2: Effect of different treatments on the number of monocyte, neutrophils, and lymphocytes.**

Parameters	PBS	200mg/Kg (High dose)	100mg/kg (Low dose)
Monocyte \ cmm	763.70±2.70	584.08±11.10	633.25±14.41
Neutrophil \ cmm	643.33±9.04	589.67±11.72	785.33±17.67
Lymphocyte \ cmm	2606.23±39.89	1043.00±5.50	2384.67±38.16

CTX, cyclophosphamide; PBS, Phosphate buffer saline; monocyte; neutrophil; and lymphocyte; ns, non-significant respectively.

As shown in Table 3, CTX treatment significantly increased the activities of AST and ALT in sera with no effect on the serum albumin level as compared to control group ( $p < 0.01$ ). Any damage to

the liver cells will result in an increase in liver enzymes (ALT & AST) [21]. The elevation of AST and ALT in our present findings is consistent with [22]. The liver is the richest source of both AST and ALT enzymes and,

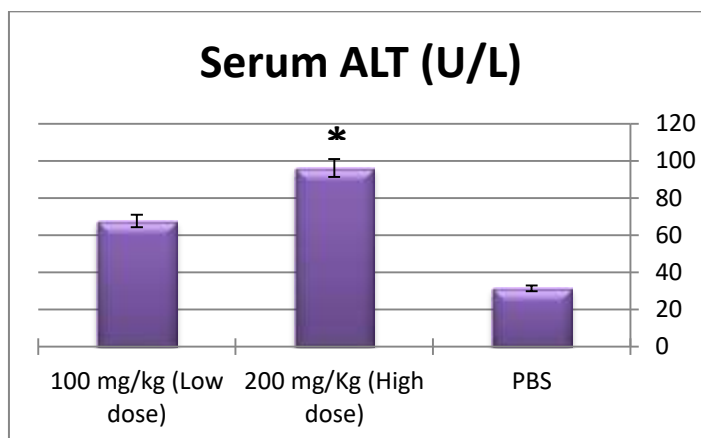
thus, the levels of both these enzymes are expected to increase as a result of damage to the liver cells [23]. That related to chemical-induced tissue injury along with hepatocellular necrosis [24]. The elevation of AST was observed in our study is consistent with the data reported by [25]. ALT is now frequently detected to estimate the degree of liver dysfunction due to CTX or advanced liver cirrhosis as well as the expectation of heart failure development [26]. In support of these reports, our data shown in Table 3 indicate that CTX treatment resulted in a significant increase in the

activities of AST and ALT in sera with no effect on the serum albumin level as compared to the control group . CPA belongs to this cytotoxic alkylating group of drugs. It is bio-activated by hepatic cytochrome P450 enzymes ensuing in the production of its two metabolites: phosphoramidomustard and acrolein. Acrolein, a byproduct metabolite of CTX, obstructs the tissue antioxidant defense system, produces reactive oxygen species (ROS), and interacts with protein amino acids causing structural and functional changes [27].

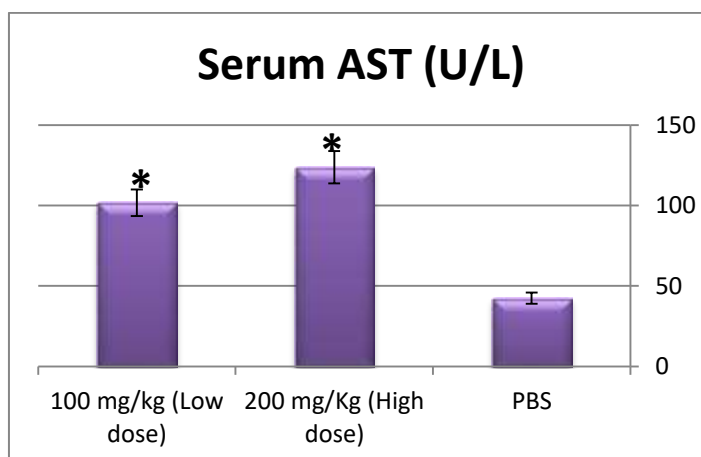
**Table 3: Effect of different treatments on serum liver function parameters.**

Parameters	PBS	200mg/Kg (High dose)	100mg/kg (Low dose)
Serum ALT (U/L)	31.39 ± 1.48	96.18 ± 2.07**	67.61 ± 1.19
Serum AST (U/L)	42.37 ± 1.46	123.78 ± 4.24**	101.67 ± 3.38*
Albumin(mg/dl)	45.01±0.91	45.76±1.10	46.35±3.27

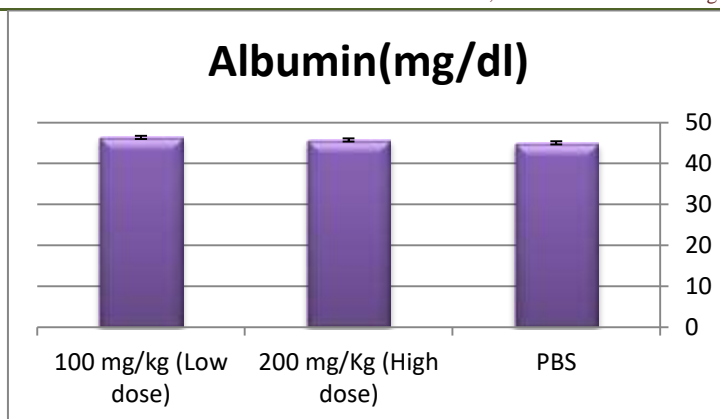
CTX, Cyclophosphamide; PBS, Phosphate buffer saline; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase and Albumin; ns, non-significant; \* and \*\* significant at P<0.05 and 0.01, respectively



**Figure 1: Statistical changes in serum ALT level after different treatment.**



**Figure 2: Statistical changes in serum AST level after different treatments.**



**Figure 3: Statistical changes in serum albumin level after different treatments.**

## REFERENCES

- G. J. Pass, D. Carrie, N. Boylan. "Role of hepatic cytochrome P450s in the pharmacokinetics and toxicity of cyclophosphamide: studies with the hepatic cytochrome P450 reductase null mouse," *Cancer Research*, vol. 65, no. 10, pp. 4211–4217, 2005.
- Ataya KM, Valeriote FA, Ramahi-Ataya AJ. Effect of Cyclophosphamide on the Immature Rat Ovary. *Cancer Res.* 1989; 49:1660-1664.
- Foley GE, Friedman OM, Drolet BP. Studies on the Mechanism of Action of Cytosine Evidence of Activation in Vivo and in Vitro. *Cancer Res.* 1961; 21:57-63.
- Poblador MS, Rojas C, Raya A, Quiralte J, Casares JA, Lancho-Alonso JL. The effects of cyclophosphamide on the prolactin cells of the normal rat. *Histol Histopath.* 1989; 4:27-30.
- Sharbaugh RJ, Grogan JB. Suppression of Reticuloendothelial Function in the Rat with Cyclophosphamide. *J Bacteriol.* 1969 Oct; 100(1):117-122.
- Lavin P, Koss LG. Effects of a Single Dose of Cyclophosphamide on various Organs in the Rat. *American Journal of Pathology.* 1971 Feb; 62(2):159-65.
- O'Connell TX, Berenbaum MC. Cardiac and Pulmonary Effects of High Doses of Cyclophosphamide and Isophosphamide. *Cancer Research* July.1974 July; 34:1586-1591.
- A. Bhattacharya, R. A. Lawrence, A. Krishnan, K. Zaman, D.Sun, and G. Fernandes, "Effect of dietary n-3 and n-6 oils with and without food restriction on activity of antioxidant enzymes and lipid peroxidation in livers of cyclophosphamide treated autoimmune-prone NZB/W female mice," *Journal of the American College of Nutrition*, vol. 22, no. 5, pp. 388–399, 2003.
- A. Stankiewicz, E. Skrzydlewska, and M. Makiela, "Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats," *Drug Metabolism and Drug Interactions*, vol. 19, no. 2, pp. 67–82, 2002.
- Brode S, Cooke A. Immune-potentiating effects of the chemotherapeutic drug cyclophosphamide. *Crit Rev Immunol.* 2008;28(2):109\_126.
- Feldman, B.F., J.G., Zinkl and N.C. Jain, (2000). *Schalm's Veterinary Hematology*. 1st Edn., Wiley, Ames, ISBN-10: 0683306928, pp: 1344.
- Burits, C.A., and E.R., Ashwood, (3rd Ed), 1999. *Tietz text book of clinical chemistry*. Philadelphia, WB Saunders, pp: 1840-1845.
- P. R., Kind, and E. J., King, (1954) "Estimation of plasma phosphates by determination of hydrolyzed phenol with antipyrin," *Jof ClinPath.* 7: 322–326.
- Huyan, X. H., Lin, Y. P., Gao, T., Chen, R. Y., and Fan, Y. M., 2011, Immunosuppressive effect of cyclophosphamide on white blood cells and lymphocyte subpopulations from peripheral blood of Balb/c mice: *Int Immunopharmacol*, v. 11, p. 1293-7.
- Grant MD, Piper M, Bohlius J, Tonia T, Robert N, Vats V et al. Epoetin and Darbepoetin for Managing Anemia in Patients Undergoing Cancer Treatment: Comparative Effectiveness Update [Internet]. Agency for Healthcare Research and Quality (US), Rockville, MD, 2013.
- Liu K, Wang GB, Cheng B, Qiu DB. Clinical comparison of GC regimen (gemcitabine and cisplatin) versus FEC regimen (fluorouracil, epirubicin, and cyclophosphamide) as neoadjuvant chemotherapy for breast cancer. *Ai Zheng* 2007;26:427–30.
- Botnick LE, Hannon EC, Vigneulle R, Hellman S. Differential effects of cytotoxic agents on hematopoietic progenitors. *Cancer Res* 1981;41:2338–42.
- Petrucci MT, Avvisati G, La Verde G, De Fabritiis P, Ribersani M, Palumbo G et al. Intermediate-dose cyclophosphamide and granulocyte colony-stimulating factor is a valid alternative to high-dose cyclophosphamide for mobilizing peripheral blood CD34+ cells in patients with multiple myeloma. *Acta Haematol* 2003;109:184–8.
- Drabeck, J. and Hofirek, B. (1977). Cytostatic effect of cyclophosphamide on blood picture in sheep. *Acta. Veterinaria. Brno.*, 46(3/4): 293-300.

20. Thatcher, N., Wagstaff, J., Willinson, P., Palmer, M. and Crowther, D. (1982). Intermittent high dose cyclophosphamide with and without prednisolone study of the relationships between toxicity response and survival in metastatic lung cancer. *Cancer*, 50: 1051-1056.
21. Cole, G. W., and Bradley, W., 1973, Hospital admission laboratory profile interpretation. The SGOT and SLDH-SGOT ratio used in the diagnosis of hepatic disease: *Hum Pathol*, v. 4, p. 85-8.
22. Senthilkumar, S., Ebenezar, K. K., Sathish, V., Yogeeta, S., and Devaki, T., 2006, Modulation of the tissue defense system by squalene in cyclophosphamide induced toxicity in rats.: *Arch Med Sci*, v. 2, p. 94-100.
23. Davila JC, Lenherr A, Acosta D. Protective effect of flavonoid on drug-induced hepatotoxicity in vitro. *Toxicology*. 1989;57(3):267\_286(30)
24. Cole GW, Bradley. Hospital admission laboratory profile interpretation. The SGOT and SLDH-SGOT ratio used for the diagnosis of hepatic disease. *Hum Pathol*. 1973;4:85\_88.(31)
25. Failure. *Diag Dis Sci*.2005;50(6):1146\_1147.(32) Senthilkumar S, Ebenezar KK Sathish V, et al. Modulation of the tissue defense system by squalene in cyclophosphamide induced toxicity in rats. *Arch Med Sci*. 2006;2:94\_100.(33).
26. Soni S, Shrivastava V. Carbendazim induced histopathological changes and some enzyme activities (GOT, GPT, ACP and ALP) in liver and kidneys of male *Rattus rattus*. *Int J Pharm Sci Health Care*. 2013;5(3):19\_35.
27. Khorwal G, Chauhan R, Nagar M. Effect of cyclophosphamide on liver in albino rats: acomparative dose dependent histomorphological study. *International Journal of Biomedical and Advance Research*, 2017;8(3):102-107.