

Original Article-BIOLOGY AND ENVIRONMENTAL SCIENCE

Study of leukopenia induced by a high doses of cyclophosphamide in mice

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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 ($20\pm 4g$) 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)

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_2O_2) during its oxidative metabolism and depresses the antioxidant defense mechanisms in the liver [8-9]. Detailed analysis of this leucopenic effect

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

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

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].

MATERIALS 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:

cyclophosphamide has dropped TLC leading to leucopenia after prolonged treatment.

was reported by [19]. The leucocytic depression (leucopenia) was also reported by [20] It may be concluded that by inhibiting haematopoiesis,

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 ($\times 10^3$)	3.8 \pm 0.48	1.80 \pm 0.48**	2.00 \pm 0.41
TEC ($\times 10^6$)	5.98 \pm 0.16	1.71 \pm 0.30**	3.60 \pm 0.32
RDW ($\times 10^4$)	20.21 \pm 0.59	15.08 \pm 0.57*	17.65 \pm 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 \pm 2.70	584.08 \pm 11.10	633.25 \pm 14.41
Neutrophil \ cmm	643.33 \pm 9.04	589.67 \pm 11.72	785.33 \pm 17.67
Lymphocyte \ cmm	2606.23 \pm 39.89	1043.00 \pm 5.50	2384.67 \pm 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: phosphoramid mustard 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 $P \leq 0.05$ and 0.01 , respectively

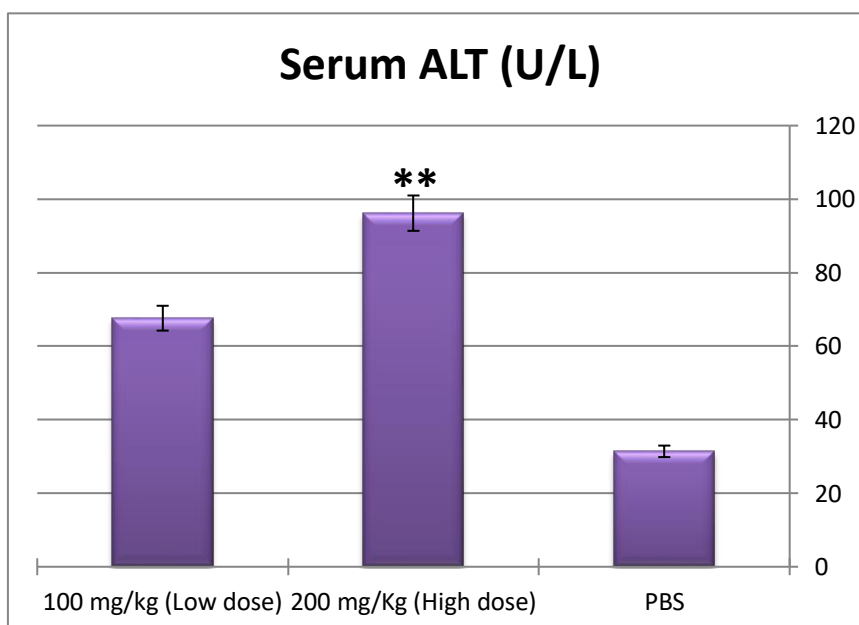


Figure 1: Statistical changes in serum ALT level after different treatment.

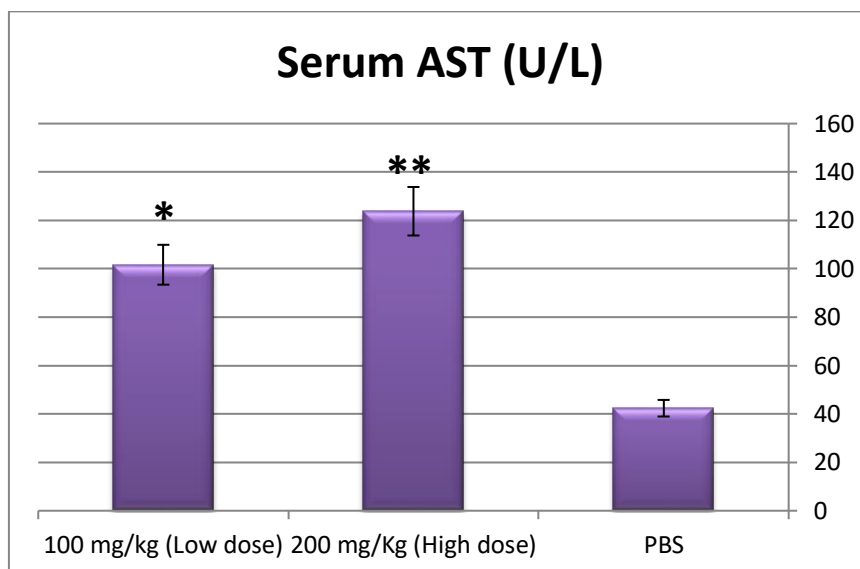


Figure 2: Statistical changes in serum AST level after different treatments.

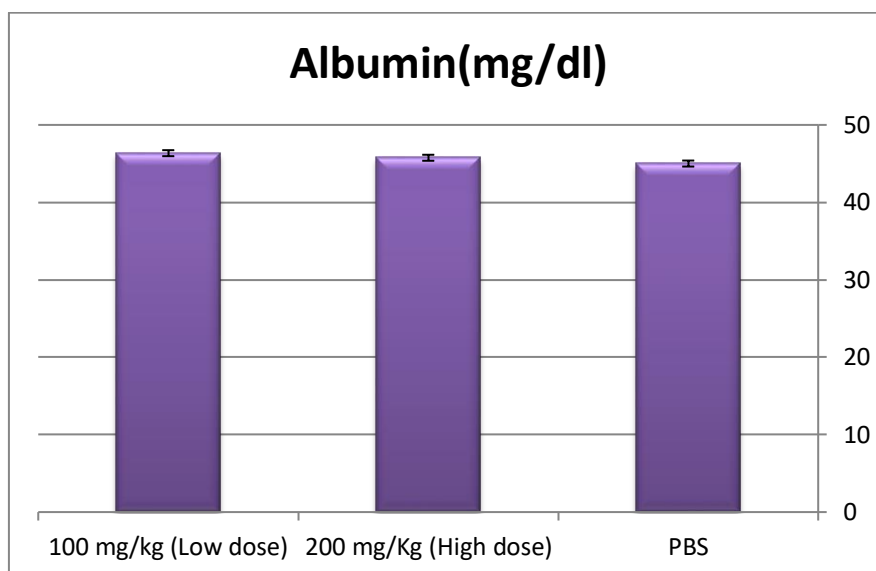


Figure 3: Statistical changes in serum albumin level after different treatments.

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