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Haematological alterations induced by cyclophosphamide administration in albino rats

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Abstract- In spite of having broad therapeutic efficacy against different types of cancer and immune related conditions, cyclophosphamide has been reported to exert toxic effects on the healthy cells with high proliferation rate. In this study, an effort has been made to investigate probable toxic effects of cyclophosphamide treatment on the functioning of bone marrow, expressed in terms of haematological changes. Experimental animals, male albino rats with average body weight of 150- 160 g were randomly divided into two groups (n= 5 in each). Vehicle control group (I) rats were treated with distilled water, whereas CYP- treated group (II) rats were intraperitoneally administered with single dose of cyclophosphamide (200 mg/kg body weight) on day 1 of the experiment. On 7th day, blood collected from rats of each group were analysed for various haematological parameters using automated haematology analyser. Data obtained were statistically analysed using Student's *t*- test. Single dose administration of cyclophosphamide resulted in a significant reduction in RBC count ($p<0.01$), haemoglobin ($p<0.001$), total WBC count ($p<0.001$), platelets ($p<0.001$), percent count of lymphocytes, PCV, MCV, MCH and MCHC as compared to non- treated group (I) rats ($p<0.01$). There was a decrease in percent count of neutrophil in rats of group II, but the change was not significant as compared to group I. Cyclophosphamide induced reduction in all types of blood cells and red cell indices can be attributed to its inhibiting effect on bone marrow activity, resulting in pancytopenia, erythrocytopenia, anaemia, panleucopenia and thrombocytopenia. So, monitoring of haematological parameters is advised for patients undergoing chemotherapy.

Key words: Cyclophosphamide, Bone marrow, Haematology, Pancytopenia, Thrombocytopenia

INTRODUCTION

Chemotherapy is a standard modality of cancer treatment that uses chemical agents or drugs to destroy cancer cells or inhibit their growth and spread.[1] In many cases, chemotherapy is combined with other treatment regimens, such as, radiation therapy and surgery to increase the effectiveness of cancer treatment. The antineoplastic drugs used in chemotherapy are used either singly or in combination with other drugs with different mechanism of action, resistance and side- effects.[2] Most of the anticancer drugs currently being used in chemotherapy are cytotoxic to normal cells at their therapeutically effective dosage.[1] Being non- selective in nature, chemotherapeutic drugs destroy rapidly dividing normal cells; such as, digestive tract, cells in bone marrow and hair follicles, leading to mucositis, alopecia, myelosuppression and immunosuppression.[2]

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Cyclophosphamide (N, N-bis (2-chloroethyl) tetrahydro-2H-1, 3,2- oxazaphosphorin-2-amine 2-oxide), an alkylating agent of Nitrogen Mustard family, has been known for its therapeutic efficacy against different types of cancer and immune related conditions, such as, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus and organ transplantation. Cyclo phosphamide is a pro- drug which is activated through its conversion into two metabolites namely Phosphoramide mustard and acrolein. Although phosphoramide mustard is responsible for its anti- tumor activity, acrolein leads to oxidative damage to cellular macromolecules such as lipids, proteins and nucleic acids.[3,4] However, cyclo phosphamide exerts its anticancer activity by interfering with DNA synthesis and division of cancer cells, its non-selectivity makes high proliferating cells of bone marrow susceptible to the damage.[3] In the present study, an effort has been made to investigate the effects of single dose administration of cyclophosphamide on various haematological parameters in male albino rats.

MATERIALS & METHODS

Experimental animals

Male albino rats with an average body weight of 150-160 g were purchased from Jaz Scientific Ranchi, Jharkhand, India. Rats were maintained under controlled and hygienic environmental conditions (22- 24^{o}C temperature and 12/12 h light/ dark cycle) with provision of ad libitum access to standard pellet diet and tap water. Animals were maintained following the provisions of institutional ethical committee of Ranchi University, Ranchi.

Experimental drug

Cyclophosphamide (Endoxan- N; 200 mg) was purchased from Azad Pharma, Ranchi, Jharkhand, India. The white crystalline powder of cyclophosphamide was dissolved in distilled water immediately before use.

Experimental design

After one week of acclimatization, rats were randomly divided into two group (n= 5 in each). Rats in group I served as vehicle control and were administered with distilled water throughout the study. Rats in group II (CYP- treated) were intraperitoneally administered with cyclophosphamide at a dose of 200 mg/kg body weight on day 1 of the experiment. Rats were maintained for 6 days. On 7th day, rats from each group were anaesthetized and blood was collected via cardiac puncture and stored in EDTA- coated vials. The dosing schedule of experimental drug to rats was based on previously published reports of Samdershi *et al.* (2019).[5]

Heamatological examination

Red Blood Cell (RBC) count, Haemoglobin (Hb) content, Packed Cell Volume (PCV), platelets, total White Blood Cell (WBC), differential percent of lymphocytes and neutrophil along with red cell indices such as, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were analysed in the blood sample collected from rats of vehicle control and CYP- treated group using automated haematology analyser.

Statistical analysis

The data obtained were expressed as mean ± Standard Deviation (SD). The difference between means of control and treatment group was analysed using Student's *t*- test and p value of $\leq$ 0.05 was considered as statistically significant.

RESULTS

Table 1 and Fig. 1 showed the effects of administration of cyclophosphamide on various haematological parameters in albino rats. Single dose administration of cyclophosphamide at the dose of 200 mg/kg body weight significantly reduced the count of RBC (49.58 %), PCV (56.26 %), haemoglobin content (57.25 %) and platelet count (68.26 %) and when compared to vehicle control group rats ($p<0.01$ and 0.001). Study of leucogram revealed a significant decline of 77.69 % in total WBC count of group II rats when compared to group I ($p<0.001$). Additionally, a significant decrease of 23.68 % was observed in % count of lymphocyte in rats of group II, treated with single dose of cyclophosphamide, when compared to non- treated group rats ($p<0.01$). There was a decrease of 20.27 % in % count of neutrophil of CYP-treated rats, but the decrease was not significant as compared to group I rats. Administration of cyclophosphamide caused a reduction in red cell indices such as, MCV (12.52 %), MCH (22.02 %) and MCHC (15.36 %), as compared to vehicle control group rats ($p<0.01$).

Table 1: Effects of single dose intraperitoneal administration of cyclophosphamide (200 mg/kg body weight) on haematological parameters in male albino rats

S. No.	Parameters	Groups		*t*-value	% change (+) Increase/ (-) Decrease
		Group I Vehicle control (Distilled water)	Group II CYP- treated (200 mg/kg body weight; i.p.)		
1.	RBC ($\times 10^6/mm^3$)	6.03 ± 1.12	3.04 ± 0.64*	4.635	- 49.58 %
2.	Haemoglobin (g/dl)	12.47 ± 2.20	5.33 ± 1.14**	5.754	- 57.25 %
3.	WBC ($\times 10^3/mm^3$)	5.38 ± 0.69	1.20 ± 0.20**	11.887	- 77.69 %
4.	Platelets ($\times 10^5/mm^3$)	4.60 ± 1.06	1.46 ± 0.51**	5.317	- 68.26 %
5.	Lymphocyte (%)	72.5 ± 3.50	55.33 ± 7.50*	4.145	- 23.68 %
6.	Neutrophil (%)	25.50 ± 6.73	20.33 ± 1.53	1.499	- 20.27 %
7.	PCV (vol %)	31.85 ± 7.95	13.93 ± 1.05*	4.468	- 56.26 %
8.	MCV (fL)	52.70 ± 2.46	46.10 ± 2.91*	3.459	- 12.52 %
9.	MCH (pg)	21.48 ± 2.12	16.75 ± 1.06*	3.981	- 22.02 %
10.	MCHC (g/dl)	39.70 ± 3.00	33.60 ± 0.62*	3.981	- 15.36 %

Values were mean ± SD (n= 5) * $p<0.01$, ** $p<0.001$ when compared to Group I.

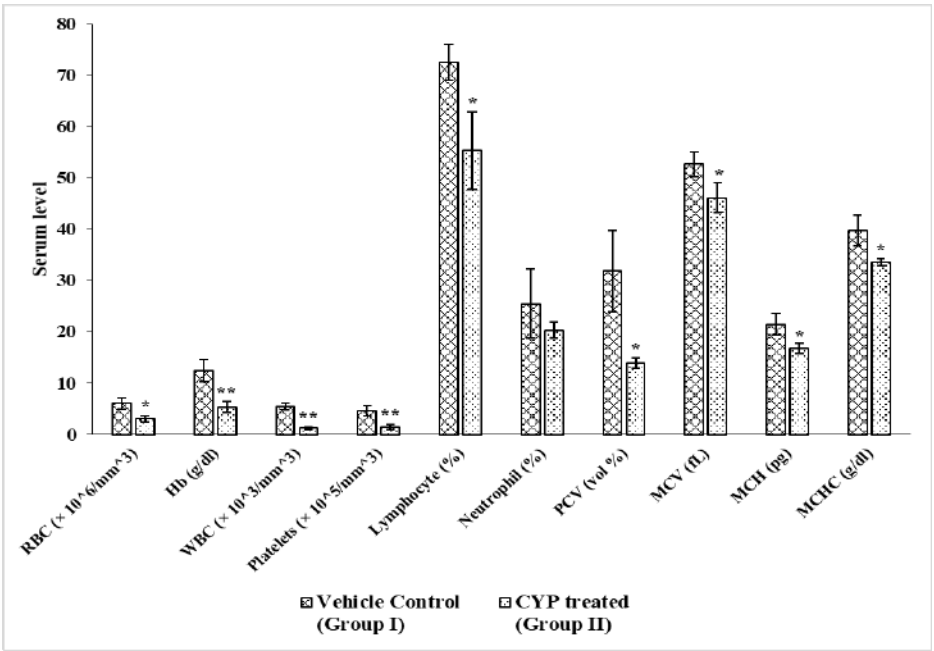

Fig. 1- Histograms showing alterations in various haematological parameters following cyclophosphamide administration in male albino rats

DISCUSSION

In the current study, single dose administration of cyclophosphamide (200 mg/kg body weight; i.p.) resulted in a significant decrease in RBC count ($p<0.01$), PCV ($p<0.01$) and haemoglobin content ($p<0.001$) of rats as compared to vehicle control group, indicating a state of erythrocytopenia and anaemia. This finding was in agreement with Shruthi *et al*., (2017)[6] who reported a significantly reduced value of RBC and haemoglobin in Swiss albino mice intraperitoneally administered with two chemotherapeutic drugs, cyclophosphamide (50 mg/kg) and cisplatin (10 mg/kg), advocating for their haematosuppressive effects on the individuals. Cyclophosphamide induced significant decline in red cell indices, such as, MCV, MCH and MCHC reported in the current study (Table 1, Fig 1) was supported by Bhalchandra *et al*., (2018)[7] who documented a significant decline in level of cell indices as well as RBC count, PCV and haemoglobin level after administration of cisplatin, a cytotoxic agent used to treat a variety of cancers, at the dose of 7 mg/kg/day for 15 days in male Wistar albino rats. Toxic effects of cisplatin treatment on RBC and haemoglobin might be due to suppression of activity of haematopoietic tissues, impaired erythropoiesis and heme biosynthesis, accelerated destruction of RBCs because of altered membrane permeability, increased mechanical fragility, and/or defective iron metabolism and haemorrhage.[7] Additionally, administration of chemotherapy has been reported to reduce erythropoietin mRNA synthesis in the liver and kidneys.[8] A lower value of MCV, MCH and MCHC in the group II rats indicated a reduction in size of RBCs, amount of haemoglobin per RBC and amount of haemoglobin per unit volume following administration of cyclophosphamide, suggesting for a clinical condition of microcytic hypochromic anaemia caused due to chemotherapy.[7,9,10]

Current study also revealed a toxic effect of cyclophosphamide on leucogram of rats. A significant decrease in total WBC count ($p<0.001$) and percent count of lymphocyte ($p<0.01$) advocated for immunosuppressive activity of the experimental drug (Table 1). The leucopenic, especially lymphopenic activity of chemotherapeutic drugs has been reported in earlier studies also.[6,7,11] Reduction in total number of WBCs might be due to increase in occurrence of infections and inflammation during chemotherapy treatment.[7] In contrary to the decline in neutrophil level of rats treated with cyclophosphamide, as reported in present study, a state of neutrophilia expressed in terms of increased level of neutrophil has been reported after administration of chemotherapeutic drugs.[11-14] Increase in neutrophil count might be attributed to the marginalisation of phagocytic cells, showing improvement in defensive responses under normal circumstances.[12] Cyclophosphamide induced decline in neutrophil count was also in line with study made by Ilamkar *et al*. (2020)[10] in methotrexate (1.75 mg/kg body weight biweekly orally) treated mice, but they reported an increment in lymphocyte percent following treatment with the drug. A significant decrease in the total WBC count and differential count of lymphocyte and neutrophil in rats of CYP- treated group might be attributed to the high dose (200 mg/kg body weight) and longer duration (6 days) of exposure to the drug. Alterations in differential count of WBC can also be related with the immune status of the individual receiving the treatment.

Our study also reported a significant decline in platelet count in CYP- treated rats (Table 1). Apart from regulation of haemostasis and thrombosis, platelets have been reported to be secondarily involved in maintenance of innate immunity, tumor growth and extravasations in the vessels.[15] A significant decline in platelet count following chemotherapy advocated for a condition of thrombocytopenia and weakened immunity in the individuals.[6,7,13,16] Bhalchandra *et al*. (2018)[7] explained that the decrease in platelet count following cisplatin treatment might be due to its bone marrow inhibiting activity resulting in decreased production of platelet or increased consumption of platelets or due to increased platelet aggregation.

Based on the findings of present study and corroborations made with previous reports, a haematotoxic effect of cyclophosphamide treatment can be recorded. As all types of blood cells are produced by haematopoietic cells present in bone marrow, a condition of pancytopenia (reduction in all types of blood cells) induced by cyclophosphamide exhibited its suppressive effects on the activity of bone marrow. Atrophic changes in the histological architecture of spleen tissues and loss of cellularity in white pulp region can provide evidences for cyclophosphamide induced perturbations in haematological parameters.[5] Wondimneh *et al*., (2021)[17]

reported a significant decrement of haematological profiles in post- chemotherapy compared to pre-chemotherapy sessions during cancer treatment. Chemotherapy induced myelosuppression as well as immunosuppression can be attributed to the loss of stem cells and inability of bone marrow to regenerate new blood cells following drug administration, mediated via induction of oxidative stress.[4,7,13,18]

CONCLUSIONS

Bone marrow suppression leading to a decline in level of haematological parameters following chemotherapy adversely affects the immune status of the individual undergoing treatment. It not only affects the effectiveness of the treatment but also renders the individual susceptible for infections from different pathogens, thereby deteriorating the quality of life and reducing the longevity. So, a better understanding of the immunosuppressive effects of the chemotherapeutic drugs can be helpful in management of its dose and duration of administration in the individual undergoing therapy and in mitigation of its adverse consequences.

List of Abbreviations

CYP- Cyclophosphamide, DNA- Deoxyribonucleic acid, EDTA- Ethylenediamine tetraacetic acid, Hb- Haemoglobin, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration, MCV- Mean Corpuscular Volume, PCV- Packed Cell Volume, RBC- Red Blood Cells, SD- Standard Deviation, WBC- White Blood Cells.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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