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Research Article

5-Fluorouracil Ameliorates the Hematotoxicity Induced by Cyclophosphamide in Male Albino Rats

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Abstract: Background: Cyclophosphamide (CPA) is a drug with a wide spectrum of clinical uses. Its effectiveness in the treatment of cancer (acute and chronic leukemias, lymphoma, and multiple myeloma) and non-malignant diseases such as rheumatoid arthritis and vasculitis has been well established. **Objectives:** The present investigation aimed to study the effect of a sub-lethal dose of the cyclophosphamide, 5-FU combination of 5-FU, and CPA on haematological parameters in the albino rats. **Materials and Methods:** Twenty-eight male adults were grouped randomly into four groups (n=5 in each group). Group I (control): Rats were injected with saline intraperitoneally at a dose of 1.0 ml/kg b.w. for 14 days. Group II cyclophosphamide (CPA): Cyclophosphamide at a dose of 10 mg/kg day by day through i.p. to rats for 14 days. Group III Fluorouracil (5-FU): 5-Fluorouracil at a dose of 10 mg/kg day by day in saline was given through i.p. to rats for 14 days. Group IV (CPA+5-FU): Rats were given CPA followed by 5-FU at a dose of 10 mg/kg per day (day by day) through i.p. to rats for 14 days. At the end of the experimental period, rats were anesthetized using light ether. Blood samples were taken for hematological evaluation. **Results:** White blood cells, hemoglobin content and red blood cell counts were significantly decline in rats treated with individual treatment with CPA and 5-FU in comparison to the control group, while the Combination antagonize the changes produced by CPA in hemoglobin and red blood cell counts. Intraperitoneal individual treatment with CPA and 5-FU in rats caused a significant reduction in the hematocrit and platelet. The reductions in these measured hematological parameters were also significantly and slightly ameliorated when the animals were given a combination of CPA and 5-FU. Cyclophosphamide and 5-FU individually reduced lymphocytes, neutrophils, eosinophils, and monocytes; while the combination of CPA and 5-FU antagonized these changes compared to CPA treated group. **Conclusion:** It could be concluded that the treatment of mammals with chemotherapy is associated with the production of free radicals that lead to hazardous alterations in hematological parameters. However, 5-FU and CPA combination could produce a significant amelioration in most cases for these changes. Future work should consider combined chemotherapy regimens, as two or more mechanisms of action of chemotherapeutic drugs could be more powerful than one mechanism. Using cyclophosphamide and 5-fluorouracil in combination may reduce cyclophosphamide's side effects when given individually.

Keywords: Chemotherapy, Cyclophosphamide, 5-Fluorouracil, Hematotoxicity, Combined Chemotherapy, Ameliorated Effect, Male Adult Rats

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1. Introduction

Cyclophosphamide (CPA), 5-fluorouracil, and methotrexate increase these patients' disease-free survival and overall survival [1]. Results of similar studies performed by

other cooperative groups have been coincident with those of the Milan trial and, additionally, the meta-analysis by the early breast cancer trialists' collaborative group has confirmed that adjuvant 5-fluorouracil improves disease-free survival as well as overall survival in all subsets of patients with operable breast cancer [2].

Arnold and Bourseaux [3] reported that the synthesis of cyclophosphamide by malignant neoplasms and treating propanolamine with N, N-bis(ß-chloroethyl) phosphamide dichloride in the presence of dioxane and triethylamine [4].

Cyclophosphamide is administered by both the parenteral and oral routes in the treatment of lymphoma, particularly in combination with vincristine and corticosteroid [5]. It is also used in the treatment of the following neoplastic diseases: mycosis fungoides, multiple myeloma, neuroblastoma, ovary adenocarcinoma, carcinoma of the breast, retinoblastoma, lung malignant neoplasms, and for bone-marrow transplantation [6-8].

It is a potent immunosuppressive agent and used to prevent rejection episodes lowing hepatic, renal, and cardiac transplantation, where there is altered immune reactivity, such as rheumatoid arthritis, Wegener's granulomatosis, autoimmune ocular diseases, children nephrotic syndrome, etc. [9].

Its effectiveness in the treatment of cancer and non-malignant diseases [10]. CPA is metabolized by cytochrome P_{450} pathways. The toxic and therapeutic effects of CPA are requirements for bioactivation by hepatic microsomal cytochrome P_{450}, mixed fmixed-function yields, and 4-hydroxycyclophosphamide that exist in equilibrium with aldophosphamide, which is degraded by β-elimination to form phosphoramide mustard and an equimolar amount of the toxic byproduct, acrolein [11, 12]. Bioconversion of CPA into these metabolites leads to the formation of high levels of reactive oxygen species (ROS), which leads to a decrease the antioxidant activity [13]. Also, excessive production of reactive oxygen species could culminate in oxidative stress [14].

5-FU is known to cause weight loss. From the literature, a wide range of 5-FU doses (20–150 mg/kg) have been administered to experimental animals [15-19]. Studies using a chronic dosing regimen to reduce tumor load have commonly used 25-30mg/kg which is used in the treatment of breast and other cancers [20-22].

5-FU has been particularly associated with patient descriptions of the cognitive side effects of chemotherapy as it has been shown that systemic treatment with this drug significantly increased the occurrence of cognitive problems when compared with treatment by local chemotherapy or local irradiation [23, 24].

The anti-tumor properties of chemotherapy depend on the proliferative activity of cells. High proliferation rates are not only a characteristic of cancer cells but also of hematopoietic cells and many epithelial tissues, which explains the common toxic side effects of chemotherapy. This results in dose-limiting toxicity and eventually in a less effective therapy [25].

Taken together, despite continuous improvements in cancer therapy and prolonged survival of treated patients, complete remissions and cure of cancer are rare and anti-cancer drugs, which selectively affect tumor cells whilst sparing normal cells, are still being searched extensively.

2. Objectives

This study aims to evaluate the effects of cyclophosphamide and/or 5-fluorouracil on haematological parameters in male albino rats.

3. Materials and Methods

The present research was conducted in the Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Egypt.

3.1. Chemicals

Cyclophosphamide and 5-Fluorouacil were purchased from Sigma Chemical Company (Saint Louis, USA).

3.2. Animals

Twenty-eight male adult albino rats with an average body weight of 180±10 g were obtained from the Faculty of Agriculture, Alexandria, and acclimatized for two weeks before the experiment. They were assigned to four groups and housed in Universal galvanized wire cages at room temperature (22-25°C) and in a photoperiod of 12h/day. Animals were provided with a balanced commercial diet.

3.3. Experiential protocol

Twenty male adult rats were grouped randomly into four groups (n=5 in each group).

Group I (control): Rats were injected with saline intraperitoneally at a dose of 1.0 ml/kg b.w. for 14 days.

Group II cyclophosphamide (CPA): Rats were injected intraperitoneally with Cyclophosphamide at a dose of 10 mg/kg day by day for 14 days [26]

Group III Fluorouracil (5-FU): Animales were injected intraperitoneally with 5-Fluorouracil at a dose of 10 mg/kg day by day [27] in saline for 14 days.

Group IV (CPA+5-FU): Rats were given CPA followed by 5-FU at a dose of 10 mg/kg per day (day by day) through i.p. to rats for 14 days.

At the end of the experimental period, rats were anesthetized using light ether. Blood samples were taken from the vena cava of the rat heart. Tubes were used to compile blood drawn from the heart directly; 1 ml was collected on sodium heparin for hematological studies.

3.4. Hematological Evaluation

One milliliter of blood was collected into heparinized sample bottles and was analyzed for hematological parameters; hemoglobin (Hb), red blood cells (RBCs), total white blood cell (WBC), hematocrit (Hct) and total platelets count using an automatic hematological assay analyzer, Advia 60 Hematology system (Bayer Diagnostics Europe Ltd, Ireland).

3.5. Statistical Analysis

The values are expressed as mean ± SEM. All values are expressed as mean±standard error of mean (SEM). Comparisons between the treatment groups and pathogenic control group were performed by analysis of variance (ANOVA) followed by Tukey- test. $P<0.05$ was considered as significant [28].

4. Results

4.1. Hematological Findings

4.1.1. WBCs and RBCs count, and Hemoglobin

In the present experimental study, it has been observed that white blood cells, hemoglobin content, and red blood cell counts significantly decline in rats treated with individual treatment with CPA and 5-FU in comparison to the control group, while the combination antagonizes the changes produced by CPA (Table 1 and Figures 1-3).

Table 1. WBCs, RBCs, and platelets count, Hb, and Hct of rat treated with cyclophosphamide and/or 5-fluorouracil.

Parameters \ Groups	Control	CPA	5-FU	CPA – 5-FU
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
WBCs count (x10³/µL)	13.64±1.65 bcd	5.10±0.50 acd	10.0±0.54 abd	3.36±0.63 abc
RBCs count (x106/µL)	7.57±0.22 bcd	4.78±0.16 acd	5.72±0.38 ab	5.52±0.47 ab
Hemoglobin concentration (g/dl)	14.42±0.66 bcd	8.94±0.40 acd	12.04±0.63 abd	11.54±0.53 abc
Hematocrit (%)	44.12±3.15 bcd	24.96±1.86 acd	34.38±0.77 ab	33.66±2.85 ab
Platelets count (10³/µl)	910.8±84.7 bc	149.2±19.1 acd	637.6±35.7 abd	876.4±31.6 bc

Significance at P < 0.05. CPA: cyclophosphamide; 5-FU: Fluorouracil, [a] Comparison of control and other groups; [b] Comparison of CPA and other groups, [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups

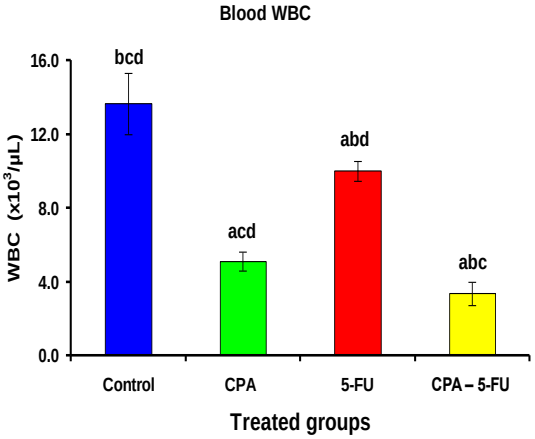

Figure 1. WBCs count (x10³/µL) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups.

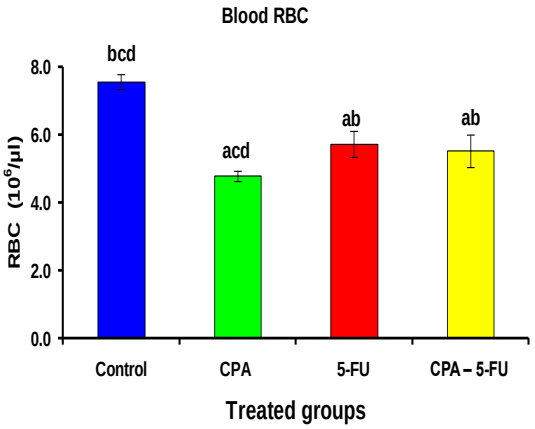

Figure 2. RBCs count (10⁶/µL) of rat treated with cyclophosphamide and 5-fluorouracil and their combination. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. a Comparison of control and other groups; b Comparison of CPA and other groups; c Comparison of 5-FU and other groups; d Comparison of CPA–5-FU and other groups.

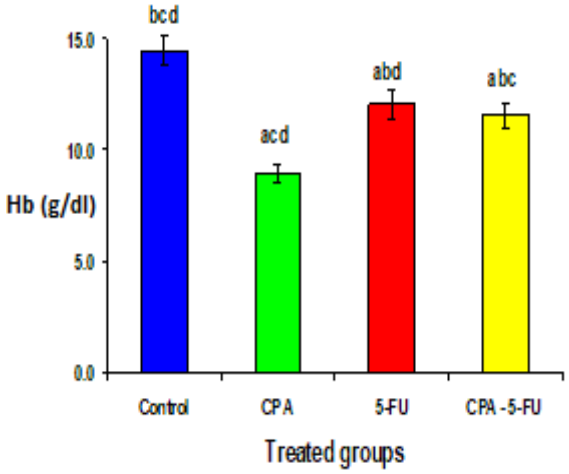

Figure 3. Hemoglobin content (g/dl) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

4.1.2. Hematocrit Values and platelets count

Intraperitoneal individual treatment with CPA and 5-FU in rats caused a significant ($p<0.05$) reduction in the hematocrit values and platelet count. The reduction in these measured hematological parameters was also significantly ($p<0.05$) ameliorated when the animal given a combination of CPA and 5-FU (Table 1 and Figures 4 & 5).

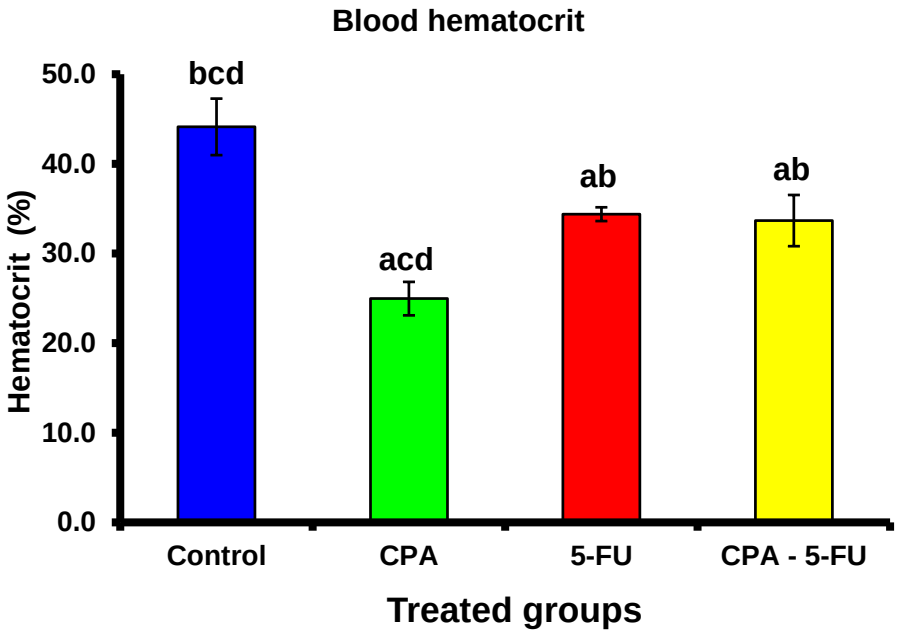

Figure 4. Hematocrit value (%) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

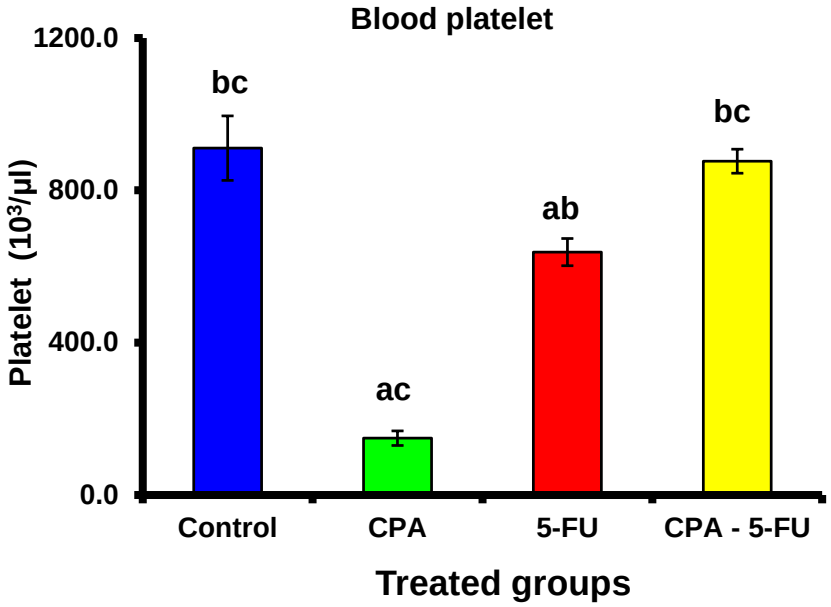

Figure 5. Platelets count (10^3/µl) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

4.1.3. Lymphocytes, neutrophils, eosinophils, and monocytes Count

Cyclophosphamide and 5-FU individually reduced lymphocytes, neutrophils, eosinophils, and monocytes; while the combination of CPA and 5-FU antagonized these changes compared to CPA treated group (Table 2 and Figures 6-9).

CPA known as an immunosuppressive drug, so the cyclophosphamide group was showed a highly significant decrease in total lymphocyte counts all over the experiment period compared with the control group.

Table 2. Lymphocytes, neutrophils, eosinophils, and monocytes Count (x10^3/µL) of rat treated with cyclophosphamide and/or 5-fluorouracil.

Groups / Parameters	Control	CPA	5-FU	CPA – 5-FU
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Lymphocytes Count (x10^3/µL)	6.22±0.24 [b]	1.62±0.15 [acd]	5.66±0.58 [b]	6.0±0.17 [b]
Neutrophils Count (x10^3/µL)	7.54±0.14 [bcd]	2.98±0.15 [ad]	3.06±0.19 [ad]	4.0±0.38 [abc]
Eosinophils Count (x10^3/µL)	0.56±0.05 [bcd]	0.36±0.05 [ad]	0.36±0.05 [ad]	0.44±0.05 [abc]
Monocytes Count (x10^3/µL)	0.46±0.05 [bcd]	0.24±0.05 [acd]	0.36±0.02 [ab]	0.37±0.03 [ab]

Significance at $P < 0.05$. CPA: cyclophosphamide; 5-FU: Fluorouracil, [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups

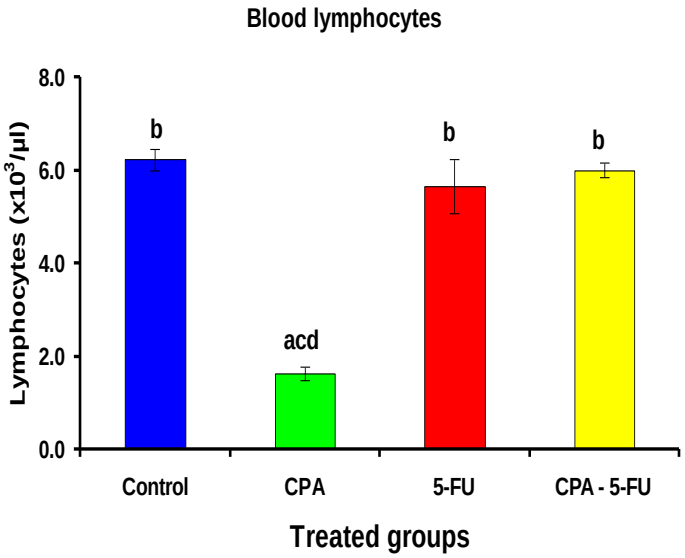

Figure 6. Lymphocytes count (x10³/µl) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

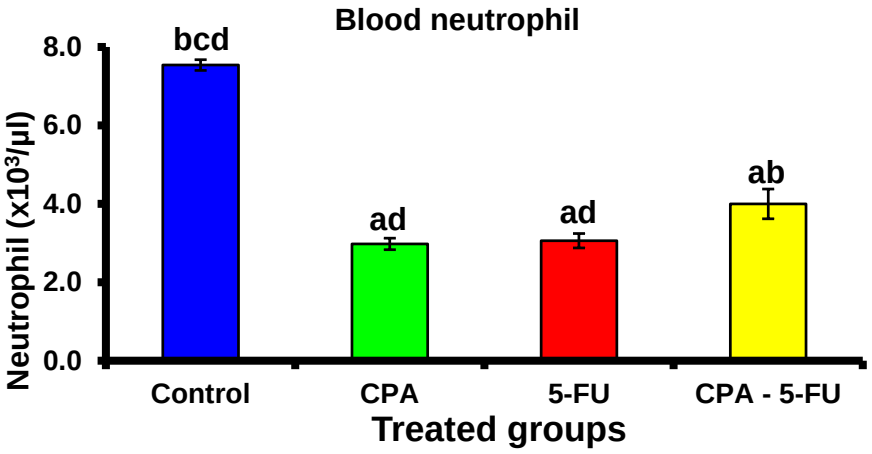

Figure 7. Neutrophils count (x10³/µl) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

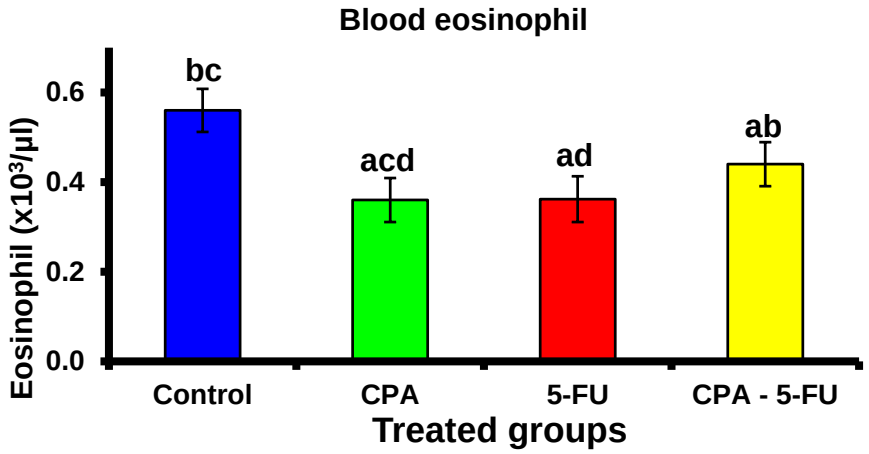

Figure 8. Eosinophils count (x10³/µl) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

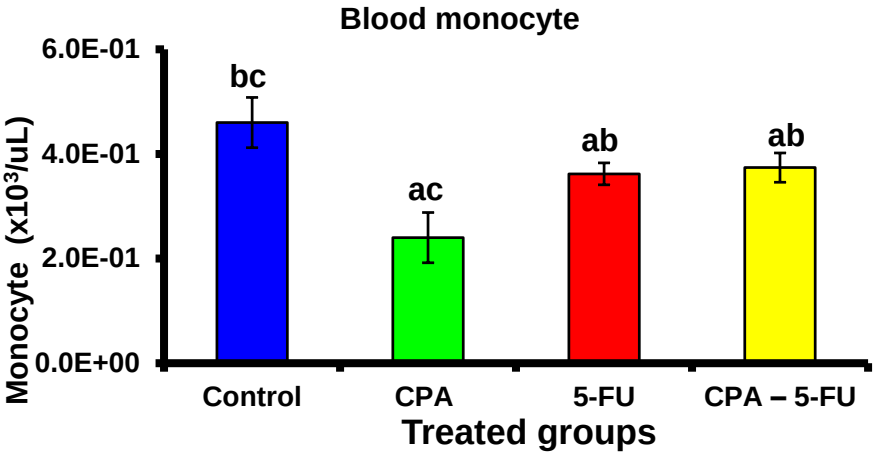

Figure 9. Monocytes count (x10³/µl) of rat treated with cyclophosphamide and/or 5-fluorouracil. Significance at $P<0.05$. CPA: cyclophosamide; 5-FU: Fluorouracil. [a] Comparison of control and other groups; [b] Comparison of CPA and other groups; [c] Comparison of 5-FU and other groups; [d] Comparison of CPA – 5-FU and other groups.

5. Discussion

Cyclophosphamide and 5-fluorouracil (5-FU) were chosen as representatives in pharmacokinetic research because they are most commonly used in cancer treatment [29, 30]. CPA is an alkylating agent, with its metabolites causing alkyl crosslinks within and between DNA strands of dividing cells, causing them to apoptosis [31]. 5-FU has been widely used in the chemotherapy of a variety of human carcinomas including head and neck, gastrointestinal tract, and breast cancer, using various schedules [32].

Our results may suggest that chemotherapy suppresses bonemarrow's ability to produce new ones, resulting in a lowering of blood cell counts which results in a decrease of Hb percentage in the blood. In connection with this, CPA altered liver and kidney functions by modulating liver enzymes [33, 34]. However, the activity of this enzyme is not limited only to the liver as it is also present in the brain, muscle, and red blood cells [35]. The cyclophosphamide-induced changes in the level of blood cells in this study were

similar to those observed in humans during treatment with this drug. The most common abnormalities found in humans are leukopenia and anemia; thrombocytopenia is observed after longer courses of treatment. Similar results were obtained in rats after a single intraperitoneal dose of CPA (50 mg/kg): Significant decreases in WBCs count and hemoglobin concentration were noted [36]. Chemotherapy-induced anemia is one of the most common side effects experienced by cancer patients, occurring in approximately 70-90% of those undergoing treatment for the disease [37].

There may be three possible causes for the decrease of the Hct during stress: increase in the volume of the plasma, loss of water in the RBCs, and haemolysis of RBCs in the bloodstream. The response to stress is characterized by hormone change (catecholamine and corticosteroid) that induced alteration in the haematological parameter [38, 39]. One mechanism is marrow suppression of megakaryocytes or possibly even generalized marrow stem cell suppression after administration of xenobiotics [40]. Another mechanism is increased platelet destruction and consumption result impairment of platelet function. Numerous xenobiotics were reported to block platelet receptor binding or to change platelet membrane charge or permeability [41]. Quantitative platelet disorders have been reported in liver disease with or without coagulation protein deficiencies [42].

Haemato-protective effect of the combination of chemotherapy may be due to the antagonistic interaction of CPA and 5-FU, since both of them produce radicals and induce lipid peroxidation to the cell wall.

Cyclophosphamide belongs to the nitrogen mustard subclass of alkylating agents. It is an immunosuppressant that alkylates DNA, thereby interfering with its synthesis and function, particularly in proliferating (also in non-proliferating) lymphocytes [43]. CPA known as an immunosuppressive drug, so the cyclophosphamide group was showed a highly significant decrease in total lymphocyte counts all over the experiment period compared with the control group. This could be attributed to severe depression of bone marrow that manifested by a significant decrease of all types of blood cells, lymphopenia, neutropenia, esinopenia, and monocytopenia. These results are in accordance with Latha and Panikkar [44] who reported leucopenia in mice treated with CPA. In addition, Holly, et al. [45] recorded leucopenia, lymphopenia, and neutropenia in female rats treated with CPA for 30 days. Unni and Martinus [46] reported significant lymphopenia in rats treated with a single dose of the CPA (250 mg/kg Bw), Zuluaga et al. [47] who reported that i.p. injection of female mice with 150 and 100 mg/kg of CPA on days 1 and 4, respectively leading to leucopenia, lymphopenia, neutropenia, and monocytopenia.

In rats, cyclophosphamide has an immunotoxic effect on lymphocytes in the spleen and blood. It has also been reported that cyclophosphamide pretreatment in rats sharply decreased the activity of all lymphoid cells, especially the CD4+ lymphocytes [48]. It was reported that both, destruction of donor antigen-stimulated T cells in the periphery, and intrathymic clonal elimination of donor reactive T cells, were essential mechanisms of cyclophosphamide-induced tolerance [49]. Phosphamide pretreatment reduces the lymphocytes in the spleen and in the blood.

6. Conclusion

It could be concluded that the treatment of mammals with chemotherapy is associated with the production of free radicals that lead to hazardous alterations in hematological parameters. However, 5-FU and CPA combination could produce a significant amelioration in most cases for these changes. Future work should consider combined chemotherapy regimens, as two or more mechanisms of action of chemotherapeutic drugs could be more powerful than one mechanism. Using cyclophosphamide and 5-fluorouracil in combination may reduce cyclophosphamide's side effects when given individually. Toxicological studies must be performed before

using drugs in combination before application. Further research is required on the toxicological impacts of drugs and pollutants mixtures.

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