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Research article

CYCLOPHOSPHAMIDE INDUCED CHANGES IN CERTAIN HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF ADULT MALE *RATTUS NORVEGICUS*.

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ABSTRACT: Background: Cyclophosphamide (CP), [2-(bis-/2-chloro-ethyl-amino)-tetrahydro-2H-1, 2, 3 oxazaphosphorine-2-oxide], is an alkylating chemotherapeutic agent, which is commonly used against malignancies, such as leukemia, lymphoma, breast, lung, prostrate, and ovarian cancer. The aim of this study is to evaluate the side effect of CP on male albino rat in response to certain hematological and hepatic and renal biochemical parameters.
Methods: In this investigation, total 20 albino rats were divided in to two groups of ten each. Group first served as control received i.p. injection of 0.9 physiological saline *fed with* standard food and water *ad libitum.* While, Group second received a single dose (0.4ml/100g b/w) through *i.p.* injection of cyclophosphamide once in a week for a period of 7 and 35 days and hematological parameters *i.e.* Hb%, total count-RBCs, total count-WBCs along with some biochemical estimations *i.e.* protein and creatinine levels in liver and kidney were quantified after 7 and 35 days of the treatments.
Results: The hematological parameters *i.e.* Hb%, total count-RBCs, total count –WBCs and protein and creatinine levels in liver and kidney were significantly lowered after 7 and 35 days treatment of cyclophosphamide. The lowering of these values was more prominent in later part of the experiment.
Conclusion: Alteration in hematological parameters after cyclophosphamide exposure may impair the functional activities of liver and kidney of male *Rattus norvegicus* by interfering enzymatic metabolic activities and protein synthesis.
Key words: Cyclophosphamide, Hematological, Biochemical, *Rattus norvegicus*.

INTRODUCTION

Cyclophosphamide (CP), an oxazophosphorine-alkylating agent, is extensively used as an antineoplastic drug in chemotherapeutic regimens of lymphoproliferative disorders, certain solid tumors and as an immunosuppressant in the treatment of autoimmune diseases such as nephritic syndrome, systemic lupus erythematosus and rheumatoid arthritis. (Morais et. al., 1999) In addition, CP is of paramount importance as an immunosuppressive agent in organ and bone marrow transplant regimens. (Demirer et. al., 1996) Apart from this, CP is used clinically to treat a wide range of cancers including malignant lymphomas, myeloma, leukemia, mycosis fungoides, neuroblastoma, adenocarcinoma, retinoblastoma and breast carcinoma. (Kovarsky, 1983; Demirer et. al., 1996) Other clinical uses for CP include immunosuppressive therapy follows organ transplants or as a treatment for autoimmune disorders such as rheumatoid arthritis, Wegener's granulomatosis and nephritic syndrome in children. (Chabner et. al., 2001) An ample literature implicate that elevated therapeutic dose of CP, caused liver disorders by the development of sinusoidal obstruction syndrome (veno-occlusive disease) and total serum bilirubin levels. (Snover et. al., 1989) Lymphocytes were more sensitive to the destructive action of alkylating agents, particularly cyclophosphamide which was more toxic to granulocytes. (Calabresi and Chabner, 1991)

It has been also reported that the chemotherapeutic drugs killed dividing cells rapidly in the body, including cancer cells and normal cells which include red blood cells. (Chakraborty et. al., 2009) Considering the above, the current study was undertaken to examine the effects of intraperitoneal dose of CP (0.4mg/100g b.wt) on certain haematological i.e. Hb%, total count RBCs, total count WBCs, and biochemical parameters *i.e.* protein and creatinine levels in liver and kidney of male *Rattus norvegicus* were done after 7 and 35 days.

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MATERIALS AND METHODS

In this investigation, 20 disease free albino rats weighing 120 ± 5 gm were acclimatized and maintained at 23 ± 2^0C temperature with a 12 hours light-dark cycle in the Animal House, Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal. The animals were fed with standard rat feed and water *ad libitum.*

Dose, preparation of drug, route and duration of administration:

Cyclophosphamide (CP) brand name-LEDOXAN known mutagenic and a pro-oxidant agent was purchased from local pharmacy market, Bhopal. CP dose (0.4mg/100g b.wt) was prepared 200mg CP was dissolved in 10 ml distilled water. Received a single dose *i.e.* 0.4mg/100g b wt of CP once in a week through intraperitoneal *(i.p.)* injection for a period of 7 and 35 days (one and five weeks).

Experimental Design:

Total 20 albino rats were divided into two groups of ten each. Group first received normal physiological 0.87% saline solution (NaCl) through intraperitoneal *(i.p.)* injection, standard food and water *ad libitum* served as the control group, while, Group second received a single dose *i.e.* 0.4mg/100g b wt of CP once in a week through *i.p.* injection for a period of 7 and 35 days and five animals from each group were sacrificed after the last dose.

Procedure:

After different intervals *i.e.* 7 and 35 days the animals were, sacrificed by cervical dislocation and blood samples were collected from every individual in a two ml sterile syringe by cardiac puncture in to a fresh EDTA blood collecting vial and hematological parameters *i.e.* Hb% quantified by using Sahli's acid haematin (Wintrobe, 1975), total count-RBCs and total count -WBCs were quantified. (Schalm et. al., 1986) While, liver and kidney were also dissected immediately, cleaned with blotting paper, weighed, homogenated in 0.85% KCl and distilled water and processed for protein and creatinine estimations respectively. (Lowry et. al., 1951; Toro and Ackermann 1975).

Statistical analysis:

Standard error of mean (SEM) were calculated and the mean values of treated as well as the control groups were compared using Student's't' test ($p < 0.05$ to 0.001). (Fisher and Yates, 1948)

RESULTS

The animals treated with CP (0.4mg/100g b.wt) for 7 and 35 days modulate Hb%, total count-RBCs and total count-WBCs as well as protein and creatinine levels in liver and kidney of *Rattus norvegicus* (Table 1 and 2). The Hb%, total count-RBCs and total WBCs-counts were significantly lowered after 7 and 35 days of the CP treatments in comparison to control groups (Table-1). In connection to this, CP also decreased protein and creatinine levels in liver and kidney in both the durations (Table-2). The above changes were more pronounced in later part of the experiment.

Table 1. Showing Hb%, Total count-RBCs and Total count-WBCs after 7 and 35 days of the cyclophosphamide exposures.

Parameters groups → ↓	Group (first) (n=10)		Group (second) (n=10)	
	Control 7 days (n=5)	Control 35 days (n=5)	Cyclophosphamide treatment after 7 days (n=5)	Cyclophosphamide treatment after 35 days (n=5)
HB% (g/dl)	14.5 ±0.288	15.453 ±0.701	9.06 ±2.333*	7.2 ±0.531***
TC-RBCs (10^6/cumm)	4.96 ±0.32	4.086 ±0.707	2.00 ±0.145***	1.98 ±0.176*
TC-WBCs (10^3/cumm)	6733.33 ±433.33	6100 ±781.024	4266.66 ±145.296***	3433.33 ±233.33**

n= number of animals.
Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits).
* = Significantly different ($p < 0.05$) from the control by Student's't' test.
** = Significantly different ($p < 0.01$) from the control by Student's't' test.
*** = Significantly different ($p < 0.00$) from the control by Student's't' test.

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Table 2. Showing protein and creatinine levels in liver and kidney of *Rattus norvegicus* after 7 and 35 days of the Cyclophosphamide exposures.

Parameters groups → ↓		Group (first) (n=10)		Group (second) (n=10)	
		Control 7 days (n=5)	Control 35 days (n=5)	Cyclophosphamide treatment after 7 days (n=5)	Cyclophosphamide treatment after 35 days (n=5)
Protein (mg/g tissue weight)	Liver	40.38 ±4.32	42.3818 ±4.507	28.387 ±0.659*	11.572 ±0.697***
	Kidney	68.46 ±2.64	70.359 ±3.776	40.103 ±2.097***	35.207 ±2.04***
Creatinine (mg/g tissue weight)	Liver	0.964 ±0.152	0.805 ±0.277	0.280 ±0.028 NS	0. 23 ±0.027 NS
	Kidney	0.834 ±0.152	0.7138 ±0.232	0.24 ±0.028 NS	0.206 ±0.096 NS

n= number of animals.
Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits).
NS = Non Significant.
* = Significantly different ($p < 0.05$) from the control by Student's't' test.
*** = Significantly different ($p < 0.00$) from the control by Student's't' test.

DISCUSSION

Cyclophosphamide is a chemotherapeutic agent, induces teratogenecity, hepatotoxicity, causes anomalies of central nervous system and skeletal system in rats, mice, rabbits, monkeys, and human. Evidences suggested that oxidative stress play a predominant a etiological role in CP induced animals. (Selvakumar et. al., 2005) Several studies indicate that CP has a pro-oxidant character, and generation of oxidative stress after CP administration leads to decrease in the activities of antioxidant enzymes and increase in lipid per-oxidation in liver, lung and serum of mice and rats. (Kaya et. al., 1999; Premkumar et. al., 2001) The property of chemotherapeutic drugs is to kill rapidly dividing cells in the body, including cancer cells and normal cells which include red blood cells. (Chakraborty et. al., 2009) CP induces leucopenia and thrombocytopenia which may be referred to its physiochemical properties such as lipophilicity, capacity to cross biological membranes and stability in aqueous solutions. In patients treated with CP, lymphocytopenia was apparent, severe within 24 hours extended for many days. Variable depression of platelet and erythrocyte counts may occur for 3 weeks after therapy. (Fisher et. al., 1993) The hematopoietic system is very susceptible to the effects of alkylating agents. Within 8 hours after administration of the sub lethal dose of CP, cessation of mitosis and disintegration of formed elements may be evident in the marrow and lymphoid tissues. CP was more toxic to granulocytes. (Calabresi and Chabner, 1991)

In the present experimental study, it has been observed that hemoglobin content and blood cell counts were significantly decline in rats treated with (0.4 mg/100g body weight) of CP in comparison to control group. Our results may suggest that CP suppresses bone marrow ability to produce new ones, resulting in lowering of blood cells counts which results in decrease of Hb percentage in the blood. In connection to this, cyclophosphamide altered liver and kidney functions by modulating liver enzymes. (Davila et. al., 1989; Abraham et. al., 2007) 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. (Ballantyne, 1988) As we know that, Protein and creatinine is an indicator for the liver and kidney function. The amount of creatinine in the blood also depends on the ability of the kidneys to excrete creatinine. Low levels of creatinine under drug exposure may be due to excretion of proteins from kidney. Along with this, it may also be due to kidney failure or impaired protein synthesis as a result of liver disorders, which may cause reduction of creatinine in liver. (Steinberg et. al., 1991) Similar results were observed in our study. It is well known that Proteins are biochemical compounds consisting of one or more polypeptides, typically folded into a globular or fibrous form in a biologically functional way, they are the building blocks. Protein has a critical physiological function.

Our study indicates that CP lowered the protein levels in *Rattus norvegicus* after 7 and 35 days of CP exposures in comparison to control groups. The reduction of protein may be due to dysfunction of hepatic protein synthesis mechanisms and the hyperactivity of hydrolytic enzymes. (Sivaprasada et. al., 1983) In accordance with our results the protein levels were also altered of exposure of alkylating agents. (Fleming, 1997) Similarly, in our experiment CP administration caused hypoproteinemia, which agrees with findings from previous studies. (Ambali, 2009) The decreased levels of protein and creatinine contents suggested that cyclophosphamide may induce hepatic and renal toxicity by interfering metabolic activities and protein synthesis. The elevation in the liver enzymes activities may be due to liver dysfunction with consequent reduction in their biosynthesis and altered membrane permeability permitting enzyme leakages into the serum. (Mansour and Mossa, 2010) The liver is susceptible to damage because of direct exposure to toxic products due to its role in the detoxification of metabolic by-products and xenobiotics. The changes occurred in liver and kidney due to cyclophosphamide administration in Rattus norvegicus may also suggested that the cyclophosphamide might be modulating the hepatic and renal functions by acting directly or indirectly on these organs and these effects are dose and duration dependant.

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