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Histopathological study on the effect of cyclophosphamide on the spleen of adult albino rat

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Abstract- Cyclophosphamide is a widely used chemotherapeutic drug which has a range of adverse effects on normal cells also. The present study was aimed to investigate the effect of cyclophosphamide on the spleen of adult male albino rat. Rats were divided into two groups of 6 individual each. Group I (Vehicle Control) rats were treated with distilled water, while group II rats were administered with a single dose of cyclophosphamide (200 mg/kg b.wt) intraperitoneally. After 6 days, rats were evaluated for spleen weight and histology of spleen. Cyclophosphamide administration significantly reduced the absolute and relative weight of spleen as compared to vehicle control group rats ($p<0.05$). Histopathological examination of spleen showed that cyclophosphamide induced marked alterations manifested by loss of clear distinction between red and white pulp, highly expanded red pulp, reduction in number and cellularity of white pulp, enlargement of central arteriole and presence of megakaryocytes in red pulp region. These findings suggested that cyclophosphamide administration may have toxic effects on the spleen resulting in immunosuppression.

Keywords- Cyclophosphamide, Spleen, Relative weight, White Pulp, Megakaryocytes, Immunosuppression.

INTRODUCTION

Cyclophosphamide is the most widely used alkylating agent in chemotherapy with a high therapeutic index and broad spectrum of activity against a variety of cancers since the late 1950's.[1] It is widely used in the treatment of several human cancers including solid tumours, lymphomas and leukaemia, as well as many non-neoplastic diseases, such as systemic lupus erythematosus and rheumatoid arthritis.[2] The anti-tumour effect of cyclophosphamide is in proportion to the dose administered, which often results in immunosuppression and cytotoxic effects.[3] As with all alkylating agents, rapidly proliferating cells are most sensitive to cyclophosphamide.[4] Being the largest secondary lymphoid organ, spleen is also greatly involved in host response against blood borne antigens.[5] An individual will be prone to infection when the spleen is damaged or removed. This fact is important to reflect that spleen acts as a suitable parameter for monitoring immune system function.[6] The current study was aimed to investigate the toxic effects of cyclophosphamide administration on weight and histopathology of spleen.

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MATERIALS AND METHODS

Male albino rats (150-160 g) were obtained from local animal supplier in Ranchi. They were housed in plastic cages and maintained under standardized environmental conditions (22-24° temperature and 12 hr light/dark cycle) with *ad libitum* access to standard pellet diet and tap water. Rats were acclimatized for 1 week before using in the experiment. Cyclophosphamide (Endoxan-N; 200 mg) was obtained from Azad Pharma, Ranchi. Rats were weighed before dividing into two groups (N=6). Group I served as vehicle control and were given distilled water. In group II (CYP-treated group), rats were administrated with a single dose of cyclophosphamide (200 mg/kg body weight) intraperitoneally. For final administration, CYP was dissolved in distilled water (1.6 ml). After administration, rats were maintained for six days with provision of food and water *ad libitum*. On 7th day, rats from both groups were sacrificed and spleens were collected. Body weight of rats was measured before sacrifice. Spleens of rats from both the groups were weighed and absolute and relative weights (% of body weight) were calculated. Small portions of spleen were cut and fixed in Bouin's fixative. Sections of about 3 µm were stained with Haematoxylin and Eosin (H & E) for histological evaluation. Photomicrography was done by using image analyser (Olympus CH20i). Obtained Data were statistically analysed by using Student's t-test and $p<0.05$ were considered significant. The values were expressed as mean ± SD (Standard Deviation).

RESULTS

Table 1: Effect of single dose administration of cyclophosphamide (200 mg/kg b.wt) on absolute and relative weight of spleen.

Sl. No.	Parameters	Group I (Vehicle Control) (Distilled Water)	Group II (CYP) (200 mg/kg b.wt)	% change Increase (+) / Decrease (-)
1.	Body Weight (g)	153.33 ± 11.54	126.67 ± 5.77*	(-) 17.39
2.	Absolute Weight of Spleen (g)	0.373 ± 0.095	0.194 ± 0.006*	(-) 47.99
3.	Relative Weight of Spleen (% of body weight)	0.253 ± 0.055	0.153 ± 0.004*	(-) 39.53

*p<0.05 Group II rats were statistically compared with Group I rats.

Table 1 showed the effect of cyclophosphamide administration on absolute and relative weight of spleen in male albino rats. In vehicle control group rats (Group I), the absolute and relative weight of spleen were 0.373 ± 0.095 g and 0.253 ± 0.055 % of body weight respectively. In group II, rats were intraperitoneally administered with single dose of cyclophosphamide (200 mg/kg body weight). After 6 days of administration, the absolute weight and relative weight of spleen was found to be 0.194 ± 0.006 g and 0.153 ± 0.004 % of body weight respectively. Cyclophosphamide administration significantly reduced the absolute weight of spleen by 47.99 % and relative weight of spleen by 39.53 % ($p<0.05$).

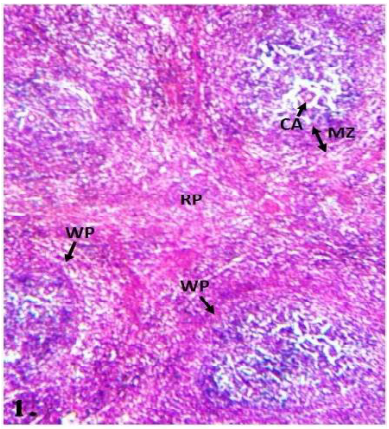

Fig 1. Photomicrograph of T.S. of spleen of vehicle control (Group I) albino rat showing distinct white pulp (**WP**) and red pulp (**RP**), well-defined marginal zone (**MZ**) and central arteriole (**CA**) (H & E; X100).

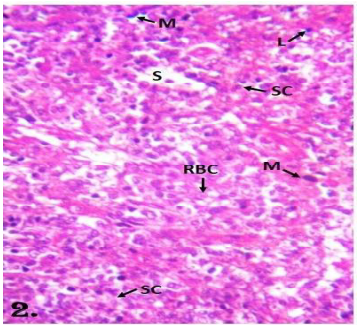

Fig 2. Photomicrograph of T.S. of spleen of vehicle control (Group I) albino rat showing normal architecture of red pulp with splenic cords (**SC**), blood sinusoids (**S**), red blood cells (**RBC**), macrophage (**M**) and lymphocytes (**L**) (H & E, X400).

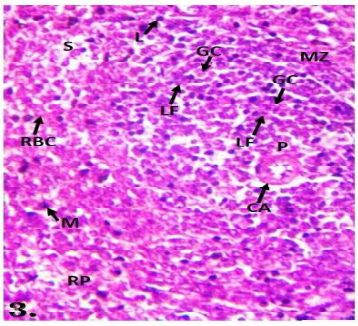

Fig 3. Photomicrograph of T.S. of spleen of vehicle control (Group I) albino rat showing normal architecture of white pulp with central arteriole (**CA**), PALS region (**P**), Lymphoid Follicles (**LF**), Germinal Centre (**GC**), lymphocytes (**L**) and well-defined marginal zone (**MZ**) (H & E; X400).

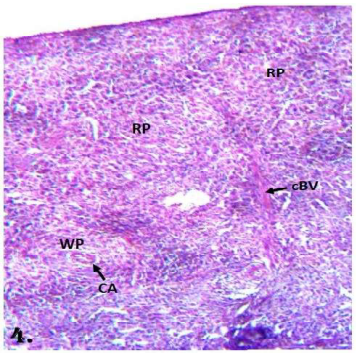

Fig 4. Photomicrograph of T.S. of spleen of cyclophosphamide-treated (Group II) rat showing indistinct differentiation of red (**RP**) and white pulp (**WP**), reduced number and size of white pulp and congestion in blood vessel (**cBV**) (H & E; X100).

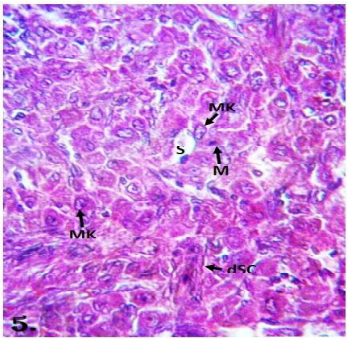

Fig 5. Photomicrograph of T.S. of spleen of cyclophosphamide-treated (Group II) rat showing large number of megakaryocytes (**MK**) but degeneration of splenic cord (**dSC**) and red pulp (H & E; X400).

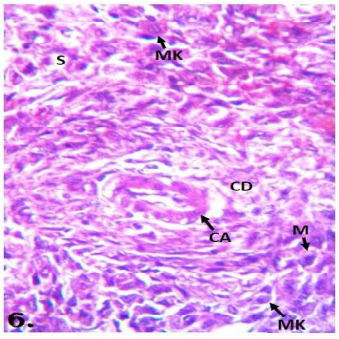

Fig 6. Photomicrograph of T.S. of spleen of cyclophosphamide-treated (Group II) rat showing enlargement and thickening of central arteriole (**CA**), areas of cellular degeneration (**CD**), loss of marginal zone and reduced cellularity in white pulp (H & E; X400).

Fig 1. showed the photomicrograph of T.S. of spleen of vehicle control albino rat. It showed normal architecture of spleen having distinct red pulp and white pulp. A well-defined, broad marginal zone was present between red pulp and white pulp; separating them into two distinct functional zones. In the spleen sections of vehicle control group rats, red pulp was composed of branching and anastomosing splenic cords and blood sinusoids in between them. The cells forming the splenic cords included lymphocytes, macrophages and other blood elements such as, red blood cells (Fig 2). H & E stained sections of vehicle control group spleen displayed multiple white pulp regions, located around an eccentric central arteriole. White pulp region was composed of periarteriolar lymphatic sheath (PALS), lymphoid follicles and marginal zone. The

lymphoid follicles consisted of a large number of lymphocytes with darkly stained nuclei. Germinal centres were also seen within some lymphoid follicles. The marginal zone was made up of few small and medium-sized lymphocytes and macrophages (Fig 3). Many small and medium-sized lymphocytes with darkly stained nuclei, macrophages and other blood elements were found scattered in the various regions of splenic pulps in the vehicle control group rat (Fig 2 & Fig 3). Rats in group II were administrated with single dose of cyclophosphamide (200 mg/kg body weight; i.p).

After six days of treatment, its effects on histopathology of spleen were studied. The spleen of cyclophosphamide-treated rat showed marked alterations within their white and red pulp. There was indistinct differentiation of red and white pulp due to loss of well-defined marginal zone. There was a visible reduction in the number and size of white pulp. Highly expanded red pulp with congested blood vessel was also observed (Fig 4). Large numbers of megakaryocytes were observed in the red pulp region of spleen of cyclophosphamide treated rat (Fig 5).

After treatment with cyclophosphamide, marked degeneration of splenic cords and loss of blood elements; such as lymphocytes, macrophage and red blood cells, were also seen in the red pulp region (Fig 5). In cyclophosphamide-treated groups, enlargement and thickening of central arteriole was observed. There was a hyaline degeneration of the wall of arteriole. There was a loss of cellularity in the PALS region. No lymphoid follicles and germinal centres were observed in the white pulp region after treatment with cyclophosphamide (Fig 6).

DISCUSSION

The present study showed that single dose administration of cyclophosphamide (200 mg/kg body weight; i.p) induced several pathological changes in the spleen of albino rat. Cyclophosphamide administration significantly reduced the absolute and relative weight of spleen as compared to vehicle control group rats ($p<0.05$).

In accordance with the present study, Kim[7] also reported a significant decrease in absolute and relative weight of spleen after intraperitoneal administration of cyclophosphamide at the dose of 150 mg/kg and 110 mg/kg. In the same context, Hou[8] also observed a significant decrease in relative weight of spleen after daily oral administration of 10 mg/kg cyclophosphamide for 30 days in wistar rats. These pathological changes in spleen were also confirmed by studying histology of spleen sections in the control and experimental group rats. In group II (Cyp-treated) rats, there was no distinct differentiation of red and white pulp due to loss of well-defined marginal zone (Fig 4).

Corroborating the present findings, Banerjee[9] reported that after administration of cisplatin, margin between white pulp and red pulp was disorganised indicating the toxic effect of the drug on the tissue architecture. After six days of intraperitoneal administration of cyclophosphamide, there was a decrease in number of white pulps in the spleen (Fig 4).

Similar effects were reported by Yoon[10] when 150 and 110 mg/kg of cyclophosphamide was used to induce immunosuppression in mice. They explained that the decrease in spleen weight observed in cyclophosphamide treated mice can be considered to be the result of the atrophic changes detected on histopathological examination.

The depletion of lymphocyte population in the red and white pulp and loss of germinal centre in the white pulp region was consistent with the previous observations that revealed decreased cellularity in the spleen of adult wistar rats given cyclophosphamide at a dose of 10 mg/kg/day for 4 weeks[11].

They also reported the congestion in blood vessels and thickening of central arteriole in agreement with the present study (Fig 4 & Fig 6). Corroborating present investigation regarding changes in red and white pulp region after treatment (Fig 5), Nabila[12] reported a highly expanded red pulps accompanied by reduced white pulps with multi small degenerated areas; as well as presence of megakaryocytes in the red pulp after treatment with colchicine.

The abundance of megakaryocytes in the red pulp region can be correlated with the red pulp increase and white pulp decrease as a result of the breakdown of normal splenic structure.[13] In a study made by Kabat-Koperska[14], a visible reduction in the white pulp and an increase in red pulp area were observed in spleen of rats while treating with combinations of immunosuppressants during pregnancy.

CONCLUSION

In conclusion, single dose administration of cyclophosphamide resulted in immunosuppression as evidenced by significant decrease in absolute and relative weight of spleen. Severe atrophic changes were also observed in the spleen sections of cyclophosphamide treated rats; such as, loss of marginal zone, reduced cellularity, congestion and presence of megakaryocytes. Therefore, a strict regulation must be taken into account before prescribing cyclophosphamide treatment.

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