

Paraquat Induced Toxicity in Spleen of Albino Mice

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Background: Paraquat is a herbicide, primarily used to control the growth of weeds and grass. This study describes the histological effects of paraquat on the spleen of the mice after single exposure to sub-lethal dose of the drug.

Methods: In this Experimental Study fifteen male mice were taken in control group (A) and twenty five in the experimental group (B). Group B received a single dose of paraquat i.e., 20 mg/kg dissolved in 0.2 ml normal saline by an intraperitoneal injection, while each animal in control group A was given an equal amount of normal saline by the same route. All animals were sacrificed on the tenth day of the experiment. The spleen from both groups was histologically examined.

Results: The result showed statistically significant difference between the thickness of trabeculae and parenchyma in the two groups. Number of inflammatory cells like neutrophils, lymphocytes and giant cells were also found to be increased in group B and the difference was statistically significant.

Conclusion: Paraquat a commonly used herbicide is easily accessible to farmers in developing countries. It has toxic effects on spleen after a single sub-lethal dose in albino mice. This is comparable to its exposure in humans and it should be carefully handled when used as herbicide by farmers.

Key words: Paraquat, spleen, toxicity.

Introduction

Paraquat is a non-selective contact herbicide; its properties were discovered in 1955 and was registered as herbicide in 1962 by ICI laboratories.¹ It is easily accessible to farmers in developing countries.² Chemically paraquat is 1, 1'-dimethyl-4, 4'-bipyridinium dichloride,³ its formula is (C₁₂ H₁₄ N₂ Cl₂). It exists in liquid form with pH range of 6.5 to 7.5, is stable in acid or neutral solutions, unstable in alkaline medium, decomposes in the presence of ultraviolet light and is inactivated by inert clays and ionic surfactants.⁴

Paraquat a highly toxic weed killer was once promoted by the United States for use in Mexico to destroy marijuana plants. Research found that this herbicide was dangerous to workers who applied it to the plants.⁵ The toxic effects of paraquat on plants are due to the production of paraquat free radicals, which, after re-oxidation with oxygen molecules, cause disorder in photosynthesis.⁶ Paraquat is taken accidentally or with a suicidal intention; it is absorbed through different routes and readily reaches all organs and tissues of the body and is not metabolized but is reduced to an unstable free radical, which, is then reoxidized to reform the cation and produce a superoxide anion. The

acceptable daily accidental intake of paraquat ion is 0.004 mg/kg body weight.⁴

Toxicity of paraquat was evaluated by dermal application of 1/2 LD50 and 1/10 LD50 for five days each week for four weeks. It was reported to induce swelling of pneumocytes, thickening of interalveolar septa and infiltration with lymphocytes and macrophages. Cerebral neurons showed degeneration and there was aggregation of pyramidal cells of hippocampus with their nuclei closely packed. The numbers of giant cells in spleen was reported to be increased.⁶

The immunotoxic effect of paraquat was investigated using Balb/c mice. Paraquat was administered at doses of 1, 0.1, and 0.01 mg/kg for 21 days. Body weight, organ weight, cellularity of spleen, delayed type of hypersensitivity response, spleen cell subtypes and lymphocyte proliferation assay were studied in various groups of animals. The results showed that high dose of paraquat could suppress both cellular and humoral activity of the immune system. Paraquat at medium dose did not show any changes in organ weight, body weight and spleen cellularity.⁷ The current project was designed to evaluate the toxic effects of paraquat on the morphology of spleen after a single sub-lethal exposure of the drug in albino mouse.

Material and Methods

Forty adult male mice of BALB/c strain, weighing 30 to 35 grams obtained from National Institute of Health, Islamabad were used for experiment. The animals were housed in stainless steel cages (five mice / cage) with wood shavings on the floor. They were fed on standard diet and fresh tap water *ad libitum*.⁸

The animals were divided in two groups. Group A served as control and consisted of fifteen mice. Group B was experimental and consisted of twenty-five animals. Each experimental animal was given a single dose (20 mg/kg) of paraquat dissolved in 0.2 ml normal saline by an intraperitoneal injection, whereas each animal in control group A was given an equal amount of normal saline by the same route. The animals were sacrificed on the tenth post-experiment day. Spleen was removed and fixed in 10 % formal saline for 48 hours. The tissues were processed for routine histology, stained with hematoxylin and eosin and studied under light microscope (Leica DM 1000).

The thickness of capsule and trabeculae of each sample was observed in four randomly selected different fields. The number of giant cells was counted in randomly selected five different fields. All measurements were made with a standardized ocular micrometer.

Microscopic data was presented as percentages and these were compared by student "t" test. The difference was regarded statistically significant if the 'p' value was < 0.05.

Results

Gross inspection of the animals showed no obvious difference between the control (A) and treated groups (B).

Histological features: Increased capsular thickness was observed in Group B (treated) but was statistically insignificant ($p = 0.18$) when compared with the control Group A (Table I).

Table I: Comparison of mean capsular thickness of spleen in control and treated groups; student –t test was applied to calculate the p-value

Study groups	Mean $\pm$ S.D (μ m)	Student "t" test	
		t-value	p-value
Control group A (15)	1.6 $\pm$ 0.50	1.37	$p =$ 0.18
Treated group B (25)	1.8 $\pm$ 0.40		

Figure in Parenthesis indicate the total number of animals in each group

Trabeculae in treated group were also found to be thickened at various places as compared to the control group (Figure I).

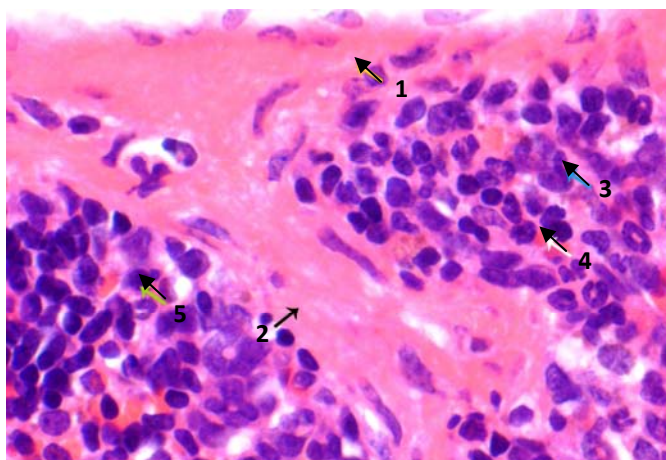


Figure I: Photomicrograph of a section of the spleen from the group B showing thick capsule (1) and trabeculae (2). Note the neutrophils (3), erythrocytes (4) and lymphocytes (5) in the area of red pulp. H&E stain. X. 400.

In histological sections of treated (A) group, there was abundant parenchymal infiltration of inflammatory cells, which consisted of lymphocytes, neutrophils, and giant cells (Figure II). The control (A) group did not reveal any inflammatory cells in the parenchyma.

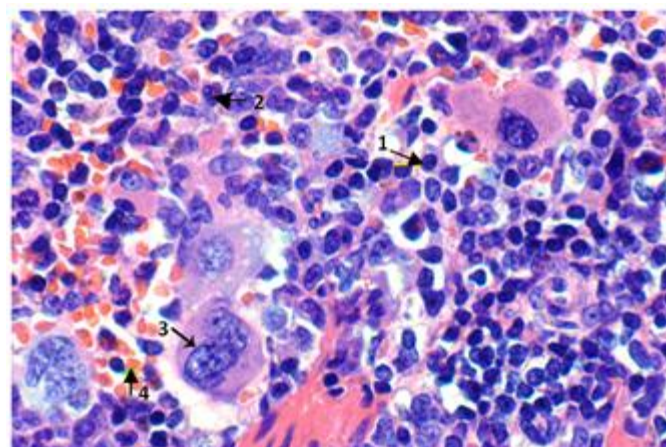


Figure: II. Photomicrograph of a section of the spleen from the treated (B) group showing infiltration of lymphocytes (1), neutrophils (2), giant cells (3) and erythrocytes (4). H&E stain. X. 400.

The increase in thickness of trabeculae in the treated group was statistically significant ($p < 0.01$) as compared to the control group (Table II).

The mean of giant cells in the treated (B) group was statistically significant ($p < 0.01$) when compared with the control (A) group (**Table III**).

Table II: Comparison of means trabecular thickness in control and treated groups

Study groups	Mean $\pm$ S.D (μm)	Student "t" test	
		t-value	p-value
Control group A (15)	1.2 $\pm$ 0.41	3.60	$p < 0.01$
Treated group B (25)	1.7 $\pm$ 0.45		

Figure in Parenthesis indicate the total number of animals in each group.

Table III: Comparison of mean giant cell count /mm² in control and treated groups

Study groups	Mean $\pm$ S.D	Student "t" test	
		t-value	p-value
Control group A (15)	1.7 $\pm$ 0.59	6.80	$p < 0.01$
Treated group B (25)	3.2 $\pm$ 0.72		

Figure in Parenthesis indicate the total number of animals in each group.

Discussion

The histological observations on spleen from treated group showed an increase in the thickness of the capsule and of the trabeculae. Mageid (1994) treated albino rats with paraquat intraperitoneally (40 mg/kg body weight) and observed paraquat induced thickening of collagen fibers surrounding the renal corpuscles.⁷ Battaller and colleagues in 2000 reported that an agricultural worker, exposed to paraquat, developed numerous skin injuries and his liver biopsy showed an increased deposition of collagen fibers in the region of the portal tract.⁸ It was also reported by Farshid et al. (2002) that peroxynitrite, a biological oxidant and cytotoxic anion, induced inflammation and injury to the lung parenchyma.⁹ Menezes et al. (2005) reported that all extensive injuries were repaired with collagen fibers (scar) irrespective of its cause.¹⁰ Increased thicknesses of the capsule and of trabeculae in our experiment may be due to increase in collagen fibers deposition induced by paraquat on comparable grounds as observed by earlier investigators.

The present study showed infiltration of neutrophils and lymphocytes, and a significant number of giant cells in the parenchyma of spleen of the treated

group. Stvrtinova et al. (1995) reported that during the inflammatory process leukocytes penetrated the endothelial wall and accumulated in the inflammatory exudate.¹¹ Luty et al. (1997) applied paraquat dermally, five days each week for four weeks to Wistar rats and observed that the numbers of giant cells in spleen were increased in the treated group as compared to those in the control group.⁵ Möst et al. (1997) reported that monocytes migrated at the site of inflammation coalesce with macrophages to form multinucleated giant cells.¹² Therefore, it was considered that infiltration of neutrophils, lymphocytes and increase in the number of giant cells, observed in the present study, could be on account of comparable reasons.

Conclusion

Paraquat a commonly used herbicide is easily accessible to farmers in developing countries. It has toxic effects on spleen after a single sub-lethal dose in albino mice. This is comparable to its exposure in humans and it should be carefully handled when used as herbicide by farmers.

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