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Paraquat Induced Hepatotoxicity in Albino Mice

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Abstract.- Paraquat is a herbicide, primarily used to control the growth of weeds and grass. This study describes the histological effects of paraquat on the liver of the mice after single exposure to sub-lethal dose of the drug. Forty male mice, fifteen control (group A) and twenty-five experimental (group B) were used. Each experimental animal received intraperitoneal injection of a single dose (20 mg/kg body weight) of paraquat dissolved in 0.2 ml of saline, while the control group was given an equal amount of saline by the same route. All animals were sacrificed on the tenth day of the experiment. The liver was removed, 3 mm^2 pieces from it were fixed in 10 % formal saline and processed for routine histological studies. Histological examination of the preparations from the treated group showed that the sinusoids and central veins of the liver were dilated and stuffed with blood cells, indicative of congestion. The liver parenchyma was infiltrated with inflammatory cells, mostly lymphocytes and macrophages. Fatty degenerative changes were also observed in the hepatocytes.

Key words: Paraquat, albino mice, herbicide, hepatotoxicity, histopathology**.**

INTRODUCTION

Paraquat is a non-selective contact herbicide discovered in 1955 and was registered as herbicide in 1962 by ICI laboratories (Paraquat-Monograph, 2003). Chemically paraquat is 1, 1'-dimethyl-4, 4'-bipyridinium dichloride (Roberts *et al*., 2002). It is used as an active ingredient in different products for protection of crops and is rapidly absorbed by green plants (Paraquat-Monograph, 2006). 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 (Luty *et al*., 1997). In animals, it is absorbed through different routes and readily reaches all organs and tissues of the body and is not metabolized; instead, it is reduced to an unstable free radical, which is then reoxidized to form a cation and a superoxide anion. The acceptable daily accidental intake of paraquat ion is 0.004 mg/kg body weight (Ashton and Leahy, 2000).

Paraquat produces both histological and functional changes in lungs, kidneys, adrenal glands, liver and myocardium, causing multi-organ failure (Paraquat-Monograph, 2003). Paraquat has several mechanisms in inducing cytotoxicity (Fukushima *et al*., 2002). In mice, when given in acute toxic dose (50 mg/kg), the animals showed signs of necrosis and inflammation of liver parenchyma (Dragin *et al*., 2006).

The effects of paraquat at a dose of 20 mg/kg were studied on creatine kinase (CK), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH) and gamma glutamyl transferase (γ-GT) activities on liver, kidney and lungs of swiss albino mice. The CK, activity was reported to decrease in liver, while GOT, GPT, LDH and γ-GT activities increased (Dere and Polat, 2001).

The toxic effects of paraquat on liver histology showed centrolobular cholestasis, hepatocellular necrosis and macrophagic infiltration of portal areas; the portal tracts were increased in size due to abundance of collagen stroma, slight infiltration by lymphocytes and leukocytes (Bataller *et al*., 2000).

Previous studies generally reported the acute toxic effects of the drug. The current project was designed to evaluate the chronic effects of hepatotoxicity of paraquat after a single sublethal exposure of the drug in albino mouse.

MATERIALS AND METHODS

Forty adult male BALB/c mice, weighing 30-35 grams, were obtained from NIH, Islamabad, and

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were housed in stainless steel cages (5 mice / cage) with wood shavings at the floor. They were fed on standard mouse diet and provided with fresh tap water *ad libitum.* They were kept under controlled condition of temperature and humidity (Mustafa *et al*., 2002).

Experimental design

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 day post-experiment. Liver was removed and its 3 mm^2 pieces were fixed in 10% formal saline for 48 hours. The tissues were processed for routine histology, stained with hematoxylin and eosin and studied with light microscope (Leica DM 1000).

Various measurements were made using an ocular micrometer that had been standardized with the help of a stage micrometer.

Statistical analysis

Microscopic data are presented as percentages. These percentages were compared according to chi-square test. The difference was regarded statistically significant if the 'p' value was < 0.05.

OBSERVATIONS AND RESULTS

Gross features

On gross examination, the liver was found to be smooth in texture; dark brown in color, possessed four lobes and was surrounded by thin layer of connective tissue capsule. Obvious gross abnormalities were not present either in control or treated group.

Histological features

In histological sections of the control group, the hepatic cords appeared to be one to two cells thick, radiating from central vein. The hepatocytes were polyhedral in shape and had a central nucleus, possessing one or two prominent nucleoli and variable number of vacuoles, scattered throughout the cytoplasm. The hepatic sinusoids were lined by discontinuous endothelial cells, contained erythrocytes and opened in the central vein (Fig. 1A).

In histological section of treated group, many hepatocytes were seen with large vacuoles, occupying most of the cytoplasm, shifting the nucleus to the peripheral part of hepatocyte (Fig. 1B).

This hepatocyte degeneration, seen in 96% of animals in the treated group was statistically significant ($P<0.0001$), as compared with hepatocytes in the control group.

The sinusoids in experimental group were filled with erythrocytes and dilated in the proximity of the central vein when compared with those in the control. The mean size of sinusoids around the central vein was 2.04 ± 0.61 and 1.40 ± 0.50 micrometers in the treated (Fig.1C) and control (Fig.1A) groups respectively; the difference was statistically significant ($P < 0.05$), when two groups were compared with each other.

The inflammatory foci consisted of lymphocytes, macrophages (Fig.1D). These were scattered through out liver parenchyma, were observed in 92% of animals in treated group and were statistically significant ($P < 0.0001$), when compared with the control group.

DISCUSSION

The histological observations on liver from treated group showed marked changes. The steatotic changes found in liver of treated group were statistically analyzed and were found to be significant. These results are similar to a previous study, which indicated that intrinsic liver injury and steatosis were common manifestations in paraquat induced liver toxicity (Tsui, 2003). Paraquat stimulates hydrogen peroxide and superoxide production in mouse liver microsome (Talcott *et al*., 1979). Paraquat has been shown to stimulate basal oxygen consumption without influencing the oxygen utilization thus having an uncoupling effect on the oxidative phosphorylation in mitochondria

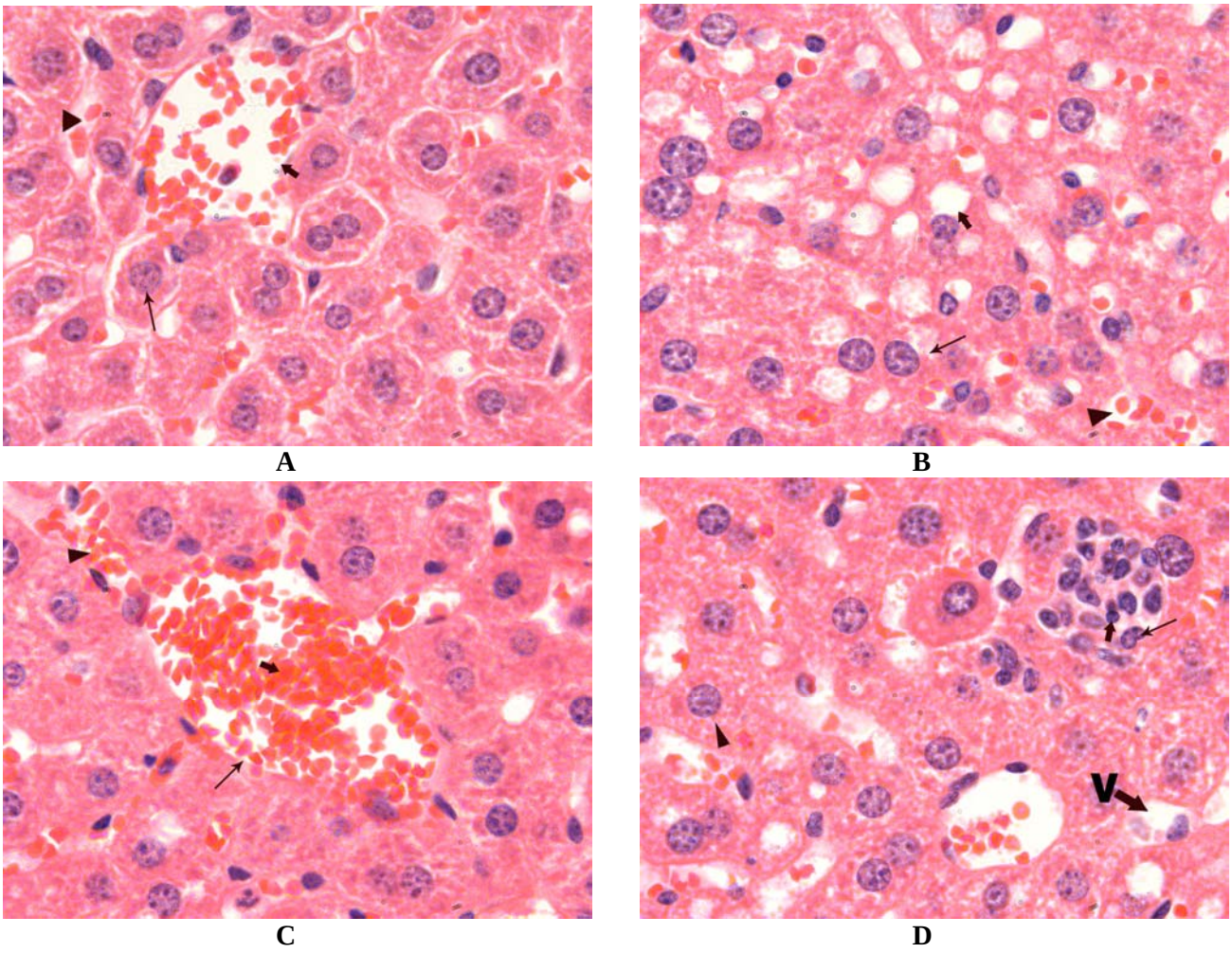

Fig. 1. Histological structure of liver Control (A) and Paraquat-Treated albino mice (B,C,D).
The control group (A) showing hepatic cords radiating from the central vein (thick arrow). Hepatocytes have centrally placed nuclei (long arrow).The sinusoids (arrow head) are seen between cords of hepatocytes. Note the presence of erythrocytes in the sinusoids; (B) treated group showing large vacuole (thick arrow) occupying most part of the cytoplasm. Variable sized small vacuoles (thin arrow) coalesce with each other in the cytoplasm of hepatocytes to form those of the larger size. Erythrocytes are seen in the sinusoids (arrow head). The treated group (C) shows central vein (thin arrow) filled with erythrocytes (thick arrow) The central vein. is surrounded by clusters of hepatocytes. Note the sinusoids are filled with erythrocytes (arrow head); (D), the treated group shows parenchymal infilteration of lymphocytes (thick arrow), macrophages (thin arrow). Hepatocytes has acidophilic cytoplasm and centrally placed nuclei (arrowhead). Large vacuole occupies most part of cytoplasm (V) comparable with hepatocyte in B straining H&E: Magnifications A, B, C, D, X630.

and changes in peroxisomes involved in the oxidation of fatty acids (Hirai *et al*., 1985; Lee *et al*., 1995; Aoyama *et al*., 1998). Paraquat also induced changes in the transcription related enzymes of liver, kidney and lungs (Dere and Polat, 2001). All these changes suggest that the enzymes responsible for esterification and entry of fatty acids into hepatocytes is impaired, thus resulting in accumulation of lipids, responsible for steatotic changes in liver of the treated animals.

Central veins and sinusoids were filled with blood cells and looked congested. Nitric oxide (NO) synthesized by the endothelial nitric oxide synthase is reported to regulate the hepatic blood flow and

vascular resistance, and plays a significant role in stabilizing the hepatic microcirculation (Day *et al*., 1999). Superoxide (O^{-}_{2}) anions generated in paraquat treated animals, react with NO and yield highly toxic peroxynitrite anion (O'Donnell and Freeman, 2001). Paraquat induced endothelial cell toxicity was attenuated by inhibitors of NO synthase, which prevent NADPH oxidation (Day *et al*., 1999)**.** Therefore, it appears that the congestion of sinusoids and central veins seen in the present study could be due to endothelial cell damage produced by peroxynitrite anion formation in the paraquat induced toxicity, leading to microcirculation failure and causing congestion in the liver.

In an *in vitro* study on Wistar rats, the pulmonary arteries of the animals were excised into small pieces of 2 mm and mounted in an organ bath containing warm gassed Krebs buffer. The pieces of the vessels were precontracted by adding a thromboxane-mimetic substance and then treated with peroxynitrite, which induced vasorelaxant effects and dilatation in blood vessels by reducing their tone (Chabot *et al*., 1997). The dilatation of sinusoids observed in the present study could possibly be explained on account of these comparable reasons *in vivo*.

The present study showed excessive infiltration of lymphocytes, macrophages in the liver parenchyma of the treated group. Leukocytes are activated by inflammatory mediators to express adhesion molecules that cause them to interact with the vascular endothelium thus allowing the inflammatory cells to penetrate the endothelial wall and accumulate in the inflamed tissue (Day *et al*., 1999). The leukocytes accumulation in the liver parenchyma of the treated animals in the present study may also be explained on account of these comparable reasons.

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