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Title of the thesis	:	"**PARAQUAT (PQ) INDUCED ULTRASTRUCTURAL AND MOLECULAR PATHOLOGY OF PULMONARY AND HEPATO-RENAL TOXICITY IN MALE ALBINO WISTAR RATS AND ITS AMELIORATION WITH VITAMIN C"**
Degree to which it is submitted	:	**MASTER OF VETERINARY SCIENCE**
Faculty	:	**VETERINARY SCIENCE**
Discipline	:	**PATHOLOGY**
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Year of Submission	:	**2019**

ABSTRACT

In the modern agriculture practices use of agrochemical agents plays pivotal role in quality and quantity of production. Among them herbicide like paraquat (PQ) is widely used for its high efficiency, low pollution and low residues in crops. The PQ may benefit the crops but causes severe fatalities in humans and animals due to lack of effective antidotes/ameliorating agents against voluntary or accidental consumption of PQ. Hence, there is a need to study the pathogenesis and effective ameliorating agent (s) against PQ induced toxicity. The present experiment was designed to study the toxico-pathological effects of PQ and its amelioration with Vitamin C in male albino *Wistar* rats. Total of 48 male albino *Wistar* rats were procured and divided into 4 groups consisting of 12 in each. The group 1 served as control whereas group 2 served as PQ toxic control (@ 40 mg/kg b.wt/day). Group 3 and group 4 rats were administered with Vitamin C (@ 250 mg/kg b.wt/day) and PQ (@40 mg/kg b.wt/day) + Vitamin C (@ 250 mg/kg b.wt/day) respectively. The experiment was carried out for a period of 21 days and six rats from each group were sacrificed on 7^{th} and 21^{st} day of the experiment.

Average body weights were recorded at weekly intervals. On the day of sacrifice, blood and serum samples were collected for analysis of haematological and biochemical parameters. Tissue samples *viz*., lungs, liver and kidneys were collected for tissue enzyme estimation, histopathological and ultrastructural studies on 7^{th} and 21^{st} day of experiment.

Significantly ($P<0.05$) decreased body weights were recorded in group 2. Haematological observations showed a significant ($P<0.05$) decrease in total erythrocyte count (TEC), total leucocyte count (TLC), Hb concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in group 2 except packed cell volume (PCV), which insignificantly elevated during 7^{th} and 21^{st} day of experiment. The serum biochemical assays revealed a significant ($P<0.05$) increase in aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and serum creatinine except total protein (TP) which significantly ($P<0.05$) reduced in group 2 on 7^{th} and 21^{st} day of experiment.

The tissue biochemical profiles showed a significant ($P<0.05$) reduction in reduced glutathione (GSH) concentration and significantly ($P<0.05$) increased

thiobarbituric acid reacting substances (TBARS) levels in selective tissues (lungs, liver and kidneys) of group 2 on 7^{th} and 21^{st} day of experiment. A significant ($P<0.05$) decrease in superoxide dismutase (SOD) activity was observed in lungs and kidneys in group 2 on 7^{th} and 21^{st} day of experiment except in liver, in which SOD activities were significantly reduced only on 21^{st} day but not on 7^{th} day of experiment. The parameters of organ morphometry in group 2 revealed a significant ($P<0.05$) increase in the absolute lung weights on day 21^{st} and significantly ($P<0.05$) decreased absolute liver weights on day 7^{th} of experiment. Absolute kidney weights were insignificantly decreased on 7^{th} and 21^{st} day of experiment. Minimal improvement was observed in all parameters in group 4 but not upto satisfactory level when compared with group 2 throughout the experimental period.

Grossly, lungs of group 2 showed diffuse haemorrhages, oedema, congestion and emphysema. Group 4, lung samples revealed focal haemorrhagic spots. Congestion and mild enlargement of liver in group 2 rats and group 4, liver revealed mild congestion only. Kidneys of group 2 and group 4 animals showed congestion.

Microscopically, group 2 lungs sections showed dilation and congestion of blood vessels, infiltration of mononuclear cells (MNCs) and polymorphonuclear neutrophils (PMNs), mild hyperplasia of bronchiolar epithelium, thickened intetstitium, diffuse type of interstitial oedema, indistinct alveolar epithelium, presence of giant cells in the interstitium and moderate to severe fibrosis in peribronchiolar area. The lung sections of group 4 exhibited reconstructive appearance of alveolar epithelium and tertiary bronchiole.

Light microscopy of group 2 liver sections exhibited congestion and distortion of central vein (CV), dilation and congestion of portal vein (PV), degeneration of bile duct epithelium, thickened wall of hepatic artery, severe dilatation of sinusoids with haemorrhages, severe periportal fibrosis. Hepatocytes are degenerated and some cells showed swollen nuclei with pyknotic changes, moderate proliferation of Kupffer cells and infiltration of dark round cells. In group 4, the sections revealed regenerating hepatic lobule with radiating appearance of cords and uniform size of nuclei.

Histologically, group 2 sections revealed variation in the glomerular morphology, severe intertubular haemorrhages, cystic dilatations of tubules, severe degeneration of tubular epithelium, focal areas of interstitial oedema, pyknotic nuclei of epithelial cells, perivascular and periglomerular fibrosis. Group 4 kidney sections showed swollen and shrunken glomeruli, focal areas of tubular epithelial degeneration, focal areas of tubular dilatations and reconstructive appearance of functional units.

The lung sections of group 2 stained with masson's trichome stain (MTS) revealed moderate to severe fibrosis of interstitium, peribronchiolar and perivascular areas. Similar changes were also observed in group rats but the lesions are mild. Group 2 liver sections stained with MTS revealed moderate to severe periportal fibrosis and the sections of group 4 exhibited mild periportal fibrosis. In group 2, the kidney sections showed proliferation of fibrous tissue into perivascular, periglomerular and intertubular spaces whereas the changes were similar but with mild degree in group 4 rats.

Histologically, the lung sections of group 2 rats stained with periodic acid Schiff (PAS) revealed hyperplasia of goblet cells (GC) in bronchiolar epithelium and excess mucous glycoprotein (MGP) secretion. Group 4 lung sections stained with PAS showed reconstructive appearance of bronchiolar epithelium and decreased MGP secretion. The liver sections of groups 1 and 3 stained with PAS showed normal topography of hepatic parenchyma. Group 2 liver sections stained with PAS revealed degeneration of glycogen in hepatocytes. The liver sections of group 4 showed similar changes to that of

group 1 and group 3. In group 2, the kidney sections stained with PAS exhibited variation in the glomerular morphology, increased thickness of tubular basal lamina, severe degeneration of tubular epithelium and cystic dilatation of tubules. Group 4 kidney sections stained with PAS revealed reconstruction of functional units with epithelial cell degeneration of proximal tubules.

Immunostaining of Bcl-2 protein in group 2 lung tissues revealed weak expression of Bcl-2 on 21st day whereas moderate expression on 7th day of experiment. Group 1 and 3 lung sections revealed strong immunostaining of Bcl-2. In group 4, the sections showed restoration of Bcl-2 concentration in alveolar epithelium in comparison with group 2.

Ultrastructurally, the cut surface of group 2 lung slices showed interstitial oedema, focal areas of collapsed and emphysematous alveoli, peribronchiolar fibrosis, oedematous septa and loss of alveolar architectural details. Group 4 lung slices showed similar lesions to that of group 2 but they are mild.

The liver slices of group 2 under scanning electron microscopy (SEM) revealed distorted cords, disrupted sinusoids with haemorrhages and round cell infiltration and fibrous tissue proliferation in sinusoids. Group 4 liver slices revealed similar mild lesions along with granular surface area of hepatocytes when compared with the lesions of group 2.

In group 2, the kidney slices under SEM showed interstitial oedema, periglomerular haemorrhages, shrunken glomeruli, periglomerular fibrosis and also swollen glomeruli with reduced Bowman's space. Group 4 kidneys revealed thickened and granular interstitium, periglomerular inflammation, loss of glomerular and tubular architectural details and mild intertubular fibrosis.

Group 2 lung ultra-thin sections revealed abnormally folded nuclei of pneumocytes I and II. Other changes noticed *viz*., vesicular nucleoli, indistinct cell organelles, swollen mitochondria, electron dense fat bodies, pyknotic nucleus, loose intercellular junction, mild margination of chromatin and severe dilatation of RER. Pericapillary and septal fibrosis were also observed. Group 4 lungs showed abnormally indented nuclei of pneumocytes, elliptical to round electron dense fat bodies and mitochondria showed cristae. Congestion of interalveolar capillaries, undifferentiated pneumocytes with various stages of nuclei, loose intercellular junctions, dilated alveoli, mild to moderate dilatation of RER, regenerating nuclei and mitochondria.

Ultra-thin sections of liver from group 2 showed swollen to shrunken nuclei of hepatocyte, severe margination of chromatin, dilatation of sinusoidal capillaries, different sizes of mitochondria with granular matrix, vesicular cytosol of hepatocytes and loss of cellular topography. In group 4, the liver sections revealed hazy nucleus, irregularly arranged swollen mitochondria with granular matrix, indistinct cell organelles, perisinusoidal fibrosis, extensively increased perinuclear space and vesicular mitochondria in cytosol.

The ultrastructure of group 2 kidney sections revealed shrunken tubules, severe dilatation of RER, swollen to vesicular mitochondria, vesicular changes of cytosol, thick intertubular junctions, swollen nucleus and thin coalesced nuclear membrane. Group 4 ultra-thin sections revealed indistinct cell organelles with uniform size of tubular epithelial nuclei and mitochondria.

In conclusion, the present study suggests that the PQ can cause early fibrosis of lungs followed by liver and kidneys. Supplementation of Vitamin C showed mild to moderate antioxidant properties against PQ induced toxic changes.