STUDIES ON THE AMELIORATIVE EFFECT OF VITAMIN E ON PARAQUAT TOXICITY IN RATS

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DECLARATION

I hereby declare that this thesis entitled **"STUDIES ON THE AMELIORATIVE EFFECT OF VITAMIN E ON PARAQUAT TOXICITY IN RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Certified that the thesis entitled **"STUDIES ON THE *AMELIORATIVE EFFECT OF VITAMIN E ON PARAQUAT TOXICITY* IN RATS"** is a record of research work done independently by **Dr. Divya V.S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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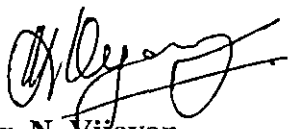

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Introduction

INTRODUCTION

Selective herbicides kill specific targets while leaving the desired crop relatively unharmed. Herbicides used to clear waste ground, industrial sites, and railway embankments are non-selective and kill all plant material with which they come into contact. Herbicides have widely variable toxicity. In addition to acute toxicity from high exposures, there is concern of possible carcinogenicity as well as other long-term problems such as contributing to Parkinson's disease.

Some herbicides cause a range of health problems ranging from skin rashes to death. The pathway of attack can arise from intentional or unintentional direct consumption, improper application resulting in the herbicide coming into direct contact with people or animals, inhalation of aerial sprays, or food consumption prior to the labeled pre-harvest interval. Under extreme conditions herbicides can also be transported via surface runoff to contaminate distant water sources. Most herbicides decompose rapidly in soils via soil microbial decomposition, hydrolysis, or photolysis. Some herbicides are more persistent with longer soil half-lives.

Paraquat (1,1'-dimethyl-4,4'-bipyridylinium) and its dichloride salt (1,1', dimethyl-4, 4'-bipyridylinium dichloride) are broad-spectrum non-selective contact plant killers that are used commercially during the past 25 years. Today, they rank among the most widely used herbicides globally and are frequently used in combination with other herbicides. Target plant species are unable to metabolize paraquat and tend to contain elevated residues; paraquat-resistant strains of terrestrial flora, whose numbers are increasing, require greater concentrations for control and may contain proportionately greater residues. Paraquat is not significantly accumulated by earthworms and other species of soil invertebrates and is usually excreted rapidly by higher animals; however, delayed toxic effects--including death of birds and mammals are common.

Paraquat is absorbed systemically in mammals, following different routes of exposure; absorption is greatest through the pulmonary route, followed by intragastric and dermal routes. Irrespective of its route of administration in mammalian systems, paraquat is rapidly distributed in most tissues, with the highest concentration found in the lungs and kidneys (Autor, 1977). The biochemical mechanism of paraquat toxicity involve generation of the superoxide anion, which can lead to the formation of more toxic reactive oxygen species, such as hydrogen peroxide and hydroxyl radical; and the oxidation of the cellular NADPH, the major source of reducing equivalents for the intracellular reduction of paraquat, which results in the disruption of important NADPH- requiring biochemical processes. The lung is the organ most severely affected in paraquat poisoning, largely due to the preferential accumulation of paraquat in lung alveolar cells. Although many organs are affected by paraquat, death is usually due to progressive pulmonary fibrosis (Suntres, 2002).

The pulmonary effects of paraquat are probably related to the conversion of paraquat to a free radical followed by conversion to a long-lived dihydroderivative, which causes transformation of normal alveolar epithelial cells to fibroblasts. This effect has been described in humans, rats, mice, guinea pigs, and dogs (Kimbrough, 1974). Other organs and systems affected by paraquat include the kidney, liver, spleen and thymus, circulatory system, gastrointestinal tract, skin, nervous system, various enzyme systems, and the eye.

Certain treatments or chemicals provide varying degrees of protection against paraquat-induced lung toxicity, but nothing is proved completely successful. Toxicity mediated by free radicals can be moderated by several cellular defense mechanisms, including superoxide dismutase, catalase, glutathione peroxidase, vitamin E, and reduced glutathione (Gabryelak and Klekot, 1985). Recognizing the fact that paraquat induces its toxic effects via oxidative stress-mediated mechanisms, innovations in the management of paraquat poisoning are directed towards the use of antioxidants.

Vitamin E is a lipid- soluble vitamin that exerts its antioxidant effects by scavenging free radicals and stabilizing membranes containing polyunsaturated fattyacids (Witting, 1980). Vitamin E is a generic term for tocopherols and tocotrienols. Vitamin E is a family of α-, β-, γ-, and δ-tocopherols and corresponding four tocotrienols. Of these, α-tocopherol has been the most studied as it has the highest bioavailability. Less expensive form of vitamin E designated as dl-α- tocopherol is also commercially available.

A detailed study on the pathogenesis of renal and pulmonary lesions in paraquat toxicity and the ameliorative effect of vitamin E will help in the management of the renal and pulmonary disorders. In view of the above facts, a study was designed utilizing rat as a model with the following objectives

(1) To asses the haemato-biochemical changes in subacute paraquat toxicity in rats.
(2) To study the pathology of renal and pulmonary lesions in subacute paraquat toxicity.
(3) To investigate the ameliorative effect of vitamin E supplementation in paraquat toxicity.

Review of Literature

2. REVIEW OF LITERATURE

Paraquat is a broad-spectrum contact weed killer and herbage desiccant that is used widely in agriculture and horticulture. Paraquat was formulated in 1882, but its herbicidal properties were not discovered until 1955. Since its introduction in the early 1960's, paraquat has been used extensively in about 130 countries. The compound belongs to the bipyridyl group of chemicals. Paraquat is a nonvolatile, ionic compound that is almost completely insoluble in organic solvents and extremely soluble in water. Paraquat is strongly adsorbed to soils and sediments and is biologically unavailable in that form; however, it is not degraded significantly for many years, except in surface soils.

2.1. TOXICOKINETICS

Daniel and Gage (1966) reported that following oral administration of paraquat, 93-95 per cent was recovered in faeces and about six per cent in urine. Thirty per cent of a dose of paraquat is present in rat faeces as metabolic products. Following subcutaneous administration, 73-96 per cent was recovered in urine and 4-17 per cent in faeces.

Sharp *et al.* (1972) first demonstrated the highest concentration in the lungs of rats intravenously dosed with paraquat and selectively retained the compound in comparison with other organs.

After oral administration of a lethal dose of paraquat to rats the plasma concentration remained relatively constant over four to thirty hours and depended on the paraquat content of the small intestine over the first 16 hours. During the first 30 hours the concentration of paraquat in the lung rose progressively above that of the plasma to levels which are known to cause pulmonary damage (Smith *et al.*, 1974).

Martin *et al.* (1981) reported that rabbit lung explants exposed to direct injury by paraquat for 18 hours at 37^0C were more resistant to oxidant injury than

rat or human lung explants. This observation suggested that the *in vivo* resistance of rabbit lung to oxidant injury is due to an inherent resistance of the lung parenchymal cells.

Nagao *et al.* (1993) suggested that paraquat was absorbed through a specialized mechanism associated with the carrier-mediated transport system for choline on the brush-border membrane. Approximately 47 per cent and 37 per cent of radioactively labelled paraquat injected into jejunal and ileal loops disappeared, respectively, after 60 minutes.

Plasma and tissue concentrations of paraquat analysed by HPLC were dose-dependent showing much higher concentration (approximately 13 times) in the lung than that in the liver whereas it was undetectable in the plasma at the same time point (Podprasart *et al.*, 2007).

2.2. MECHANISM OF ACTION

Gardiner (1972) suggested that the disordered pulmonary function and production of pulmonary oedema fluid in acute paraquat poisoning were attributed to interference with surfactant activity in the lung.

After intravenous injection of ^{14}C paraquat (20 mg/kg body weight), tissue localization was preferential in lungs of rats as well as rabbits, but the accumulation of paraquat into the lung cannot be explained as a consequence of the binding of paraquat to the tissue (Ilett *et al.*, 1974).

Bus *et al.* (1976) conducted studies on paraquat toxicity and demonstrated that the *in vivo* toxicity of paraquat may be mediated through lipid peroxidation of cell membranes. According to them paraquat toxicity appeared to result from cyclic reduction oxidation of the herbicide with subsequent generation of superoxide radicals. Superoxide radicals may non-enzymatically dismutate to singlet oxygen, which reacts with unsaturated lipids in cell membranes to form lipid hydroperoxides which initiates the membrane destructive process. They also

reported that paraquat toxicity in mice was increased by exposure to 100 per cent oxygen and by deficiencies of the antioxidants selenium, vitamin E, or reduced glutathione.

Bus and Gibson (1984) identified that pulmonary toxicity of paraquat resemble the toxicity of several other lung toxins, including oxygen, nitrofurantoin and bleomycin. Paraquat toxicity resulted in depletion of intracellular NADPH which was necessary for maintenance of glutathione in the reduced state.

Smith (1985) investigated that, with toxic levels of paraquat in the cell, compensatory biochemical processes are insufficient to maintain levels of NADPH consistent either with cell survival or with the ability to detoxify hydrogen peroxide or prevent lipid peroxidation.

Ogata and Manabe (1990) performed biochemical and morphological studies of rat lung to determine the role of lipid peroxidation in the *in vivo* toxicity of paraquat by administering two injections of 20 mg/kg paraquat every other day and observed that the lipid peroxide concentrations and the number of macrophages in the lung reached maximum on the seventh day post injection, when the damaged alveolar surface had been mostly repaired by regenerative pneumocytes. These results suggested that lipid peroxidation is a relatively late event in the *in vivo* paraquat-treated lung and that the delayed increase of lipid peroxides in the lung occurs from the phagocytic activities of macrophages rather than from toxic cell injury.

Bianchi *et al.* (1993) studied the effect of paraquat on the expression of the neutrophil chemotactic cytokine, interleukin-8, by human peripheral blood mononuclear cells and their findings suggested that Interleukin-8 might be involved in the pulmonary effects of paraquat and that its production might be stimulated following an oxidative insult and might clarify the pathogenic mechanisms of oxidant-induced pulmonary fibrosis.

Yamada and Fukushima (1993) suggested that paraquat was reduced by NADH-ubiquinone oxido-reductase, but there was difference in the lipid peroxidation by the paraquat radical between rat lung and heart, that is lipid peroxidation increased in lung but not in heart.

Bainy *et al.* (1994) studied the influence of lindane and paraquat on oxidative stress-related parameters of the red blood cell *in vitro* and found out that red blood cells exposed to paraquat exhibited a concentration dependent decrease in the t-butyl hydroperoxide-induced oxygen consumption and increments in either the induction period or in the activity of catalase and glucose-6-phosphate dehydrogenase, with no changes in superoxide dismutase activity and a small decrement in that of glutathione peroxidase activity while lindane addition did not interfere with the oxidant status of the erythrocyte.

Fukushima *et al.* (1994) studied the effects of acute paraquat exposure on mitochondrial function in rat lung and the results indicated that the cytotoxicity via mitochondria dysfunction by acute paraquat exposure might be caused by complex 1 toxicity following lipid peroxidation of mitochondrial inner membrane.

The induction of peroxide metabolic enzymes by paraquat followed the increase of thiobarbituric acid reaction substance, an indicator of lipid peroxide concentration (TBARS) in the liver and kidney of mice, but in lung, the enzyme induction without the increase of TBARS was observed (Takenaka and Goto, 1994).

Tawara *et al.* (1996) suggested that paraquat after crossing the blood-brain barrier might be reduced to the radical in rat brain, which may damage the brain tissue, especially dopaminergic neurons in striatum after acute paraquat exposure.

2.3. HAEMATOBIOCHEMICAL ALTERATIONS

Paraquat , when administered intraperitoneally to mice, at 30 mg per kg body weight, decreased concentrations of the water-soluble antioxidant glutathione in liver and lipid soluble antioxidants in lung. Rats exposed to 100 ppm paraquat in the drinking water for three weeks had significantly elevated activities of lung glucose-6-phosphate dehydrogenase and glutathione reductase (Bus *et al.*, 1976).

A marked diuresis, albuminuria, glycosuria, and an increased plasma urea concentration occurred 6-24 hours after paraquat (680 micromoles per oral, or 108 micromoles, subcutaneously) administration (Lock and Ishmael, 1979).

In the paraquat treated group of one-day old rats, the total lung protein increased by about 18 per cent and the enzyme phosphatidic acid phosphatase decreased about 30 per cent (Hunter and Prahlad, 1981).

Hoffman *et al.* (1987) observed elevation of plasma LDH activity and total protein concentration and decrease in plasma alkaline phosphatase activity in American kestrel nestlings receiving 60 mg/kg of paraquat dichloride for 10 days.

The effect of paraquat on lung subcellular calcium transport was studied in male Sprague-Dawley rats with a high dose of 30 mg/kg body weight given intraperitoneally. A significant decrease in the lung mitochondrial and microsomal calcium uptake at 24, 48 and 72 hours after paraquat administration was noticed (Agarwal and Coleman, 1988).

Rios *et al.* (1995) observed that, in germ cells paraquat induced an increase of sperm shape abnormalities when the animals were treated with paraquat at three stages of cell development: Spermatozoa, spermatid and preleptotene spermatogonial cells.

Effects of paraquat on the substantia nigra of the male Wistar rats were studied pharmacologically by an intracerebral injection of paraquat. Neurochemically, paraquat caused dose-dependent depletion of dopamine in the ipsilateral striatum starting two weeks after treatment; this effect was long-lasting and irreversible (Liou *et al.*, 1996).

Paraquat increased the pulmonic, cardiac and hepatic concentration of hydrogen peroxide, conjugated dienes, lipid peroxides and malonyldialdehyde but it did not noticeably increase the concentration of these substances in the kidney (Anguelov and Chichovska, 2004).

Decreased Hb, TLC, total serum protein, creatinine and increased SGOT, total bilirubin and BUN level were noticed in calves fed with paraquat sprayed grass. There was significant increase in neutrophil together with significant relative decrease in lymphocytes (Tamuli *et al.*, 2004).

Urban *et al.* (2005) noticed a genotoxic effect of paraquat even after exposure via dermal application. Paraquat at dose levels of 6, 15 and 30 mg/kg body weight was given to rats via the dermal route and number of micro-nucleated polychromatic erythrocytes was found to be increased in a dose dependent manner.

Sato *et al.* (2006) examined the lipid peroxide levels of homogenate prepared from the lungs, livers, kidneys and brains of 48 male mice treated with 30 mg/kg paraquat dichloride. A significant increase in the lipid peroxide level was identified only in the liver.

Lamfon and Al-Rawi (2007) reported that rats intoxicated with paraquat at a dose level of 1/36 LD50 (3.46 mg/kg BW) three times per week for three weeks caused marked elevation in serum creatinine and blood urea nitrogen.

Hypobilirubinemia and hypoalbuminemia were observed in male Wistar rats treated with paraquat, at the dose range 4.0-6.0 mg/kg body weight for seven consecutive days (Podprasart *et al.*, 2007).

Ray *et al.* (2007) conducted a study on the anti-oxidant defense system of male albino rats after administering paraquat dichloride and observed that glutathione level in blood cells, liver, lung and kidney was decreased and malondialdehyde formation was increased in a dose and time dependent manner.

In experimentally induced acute paraquat toxicity in calves and goats, haemoglobin concentration, total leukocyte counts and neutrophils were increased, while lymphocyte count was decreased. The SGOT, BUN and total serum protein levels increased significantly in both species (Tamuli et al., 2009).

2.4. GROSS PATHOLOGY

Hearn and Keir (1971) observed nail damage in 55 persons due to leakage of diluted paraquat from the knapsack sprayer. The commonest lesion seen was transverse white bands of discoloration, but loss of nail surface, transverse ridging, gross deformity of the nail plate, and loss of nails also occured. The index, middle, and ring fingers of the right hand were predominantly affected.

Marked pulmonary congestion was found in female Sprague-Dawley rats treated with 25mg paraquat dichloride per kg body weight intravenously (Thurlbeck and Thurlbeck, 1976).

The body weight was slightly decreased in one-day old rats treated with 25 mg paraquat per kg body weight, but the lung weight was not significantly lower than those of the control (Hunter and Prahlad, 1981).

Tabataa *et al.* (1999) observed that in conjunction with lung involvement a pronounced degeneration in skeletal muscle of a man who died on the 14^{th} day after the intentional ingestion of paraquat.

Pegu *et al.* (2002) conducted experimental paraquat toxicity in goats with special reference to the respiratory system and gross lesions observed in the lungs included mild to severe congestion covering most part of the lungs along with profuse white froth in the bronchi and trachea. Varying degree of emphysema was also reported.

Tamuli *et al.* (2003) conducted a study to investigate the pathological alterations induced by ascending dose of paraquat through oral route in calves (LD50: 50-75 mg/kg body weight) and observed that the calves dosed with 50 and 75 mg paraquat dichloride/kg body weight died on 14^{th} and 2^{nd} day respectively and calf dosed with 25 mg/kg body weight survived. The post mortem examination revealed moderate to severe congestion, haemorrhages and varying degrees of emphysema in the lungs, slight enlargement of liver, dilatation of blood vessels in brain and mild to severe congestion of the small intestinal and abomasal mucosa.

Tamuli *et al.* (2004) studied the toxicity of paraquat sprayed grass in calves and the clinical signs noticed were lacrimation, frequent urination, frothy salivation, dehydration and emaciation along with muscular tremor and staggering gait. Gross lesions noticed were congestion and emphysema in the lungs, focal necrotic lesions in the liver, congestion and haemorrhages in the cerebral cortex, heart, kidney and small intestines.

Grant *et al.* (2007) first reported cerebral changes in eight people who died of paraquat poisoning. Oedema and haemorrhage were the most consistent and significant findings: they suggested that paraquat may damage the cerebral blood vessels.

Podprasart *et al.* (2007) reported that multi-low dose of paraquat might affect certain synthetic function of the liver or activity of some hepatic xenobiotic-metabolizing enzymes without significant alteration in the liver

morphology by administering paraquat at the dose rage of 4.0-6.0 mg/kg body weight per day subcutaneously to male Wistar rats for seven consecutive days.

2.5. HISTOPATHOLOGY

Butler and Kleinerman (1971) observed atrophy of the thymus and minimal renal tubular and focal peritoneal changes in rabbits treated with 25 mg per kg body weight of paraquat intraperitoneally. The delayed pulmonary changes produced in man and other species by paraquat were not found in rabbits.

Renate and Kimbrough (1974) reported that paraquat caused the following sequence of events in the lung of man : vacuolation and degeneration of the alveolar epithelium, edema of the alveoli, widening of the basement membranes, and appearance of the red blood cells in the alveoli. These events were followed by infiltration of the lung with inflammatory cells, fibrosis, epithelial proliferation, and accumulation in the alveoli of electron dense material that resembles lamellar bodies and surfactant. In few rats treated with 500 ppm paraquat daily in the diet, they observed focal areas of consolidation containing proliferated epithelial cells surrounded by inflammatory cells and fibroblasts in lung parenchyma.

Smith and Heath (1975) described in the pathogenesis of paraquat in lung of man involves a destructive phase and a proliferative phase, and these two stages appear to be independent of one another. The destructive phase consists of swelling and fragmentation of the alveolar epithelium followed by alveolar oedema and an acute inflammatory exudate. In rats the proliferative phase commences with an infiltration into the alveolar spaces of profibroblasts which then mature via a series of stages into mature fibroblasts to produce a diffuse intra-alveolar fibrosis.

The pulmonary ultrastructure of the late stage of a case of human paraquat poisoning revealed reduced number of alveoli in the paraquat patients and they

were filled with edematous proteinaceous plasma-like fluid containing erythrocytes, macrophages, leukocytes, fibroblast-like cells, platelets and fibrin. These alveoli were lined by granular pneumocytes. Interstitial areas were greatly thickened with collagen, fibrin, mature fibroblasts and many leukocytes (Dearden *et al.*, 1978).

Histological examination of the kidneys showed mild hydropic degeneration of the proximal convoluted tubules in rats treated with 680 micromoles of paraquat per oral or 108 micromoles, subcutaneously (Lock and Ishmael, 1979).

Burk *et al.* (1980) found that paraquat caused liver and kidney necrosis in selenium deficient rats.

Hunter and Prahlad (1981) identified an increase in the thickness of the alveolar wall with much intra-alveolar infiltration of cells and cell debris in one-day old rats treated with paraquat.

Palmeira *et al.* (1994) investigated the cytotoxic effects of the paraquat on freshly isolated rat hepatocytes and found out that paraquat caused a dose and time dependent cell death accompanied by depletion of intracellular glutathione and mirroring increase of oxidized glutathione.

Intranigral injection of 2 mg of paraquat produced marked loss of Nissl substances and prominent glial reaction in the substantia nigra, while 3 microgram of paraquat caused a severe loss of neurons in rats (Liou *et al.*, 1996).

Kao *et al.* (1999) reported predominant changes in acute paraquat intoxications as infiltration of inflammatory cells and haemorrhage in the interstitium and alveolar air spaces, detachment of alveolar epithelial cells from the basement membrane and total obliteration of alveoli resulting in pulmonary fibrosis.

Pegu *et al.* (2002) conducted experimental paraquat toxicity in goats with special reference to the respiratory system and histopathological examiantion of lungs revealed congestion and varying degrees of emphysema. Diffuse haemorrhages were observed in the interstitial perivascular areas and at places, areas of oedema revealing homogenous pinkish staining material in some alveoli were observed.

Tamuli *et al.* (2003) reported the pathological alterations induced by paraquat through oral route in calves. Microscopically, in the lung, proliferation of fibroblasts around the dilated and congested blood vessels and hyperplasia of bronchial epithelium were observed. Marked degenerative changes, like cellular swelling, fatty changes and focal necrosis were observed in the liver. The sinusoids and central veins were severely congested. Kidneys showed dilatation of blood vessel with severe congestion and haemorrhages in the interstitial spaces. Degeneration of the lining epithelium of the tubules and presence of eosinophilic homogenous masses in the lumina were observed. Neuronal degeneration, neuronophagia, satellitosis and gliosis were also observed. Intestinal sections revealed infiltration of neutrophils in the submucosa and lamina propria and highly congested blood vessels.

In a study conducted on the toxicity of paraquat sprayed grass in calves (Tamuli *et al.*, 2004) noticed severe congestion, focal haemorrhages, emphysema and thickening of interstitial spaces in lungs. Microscopically liver and kidneys showed various degenerative changes, focal necrosis and congestion. Intestinal mucosa showed varying degrees of congestion and haemorrhages.

Dede *et al.* (2007) studied the toxicological effects of paraquat on the histology of the stomach, small intestine and testis of male albino rats and histopathological examination of the organs studied revealed mild mucosal ulceration, muscular coat atrophy, stromal oedema and tubular hyalinization in the stomach which were dose dependent. The small intestine showed mucosal ulceration, loss of villi, luminal and stromal oedema and glandular necrosis. The

testis had classical central fibrosis, cellular polarization, tubular disorganization, necrosis and lack of mitotic figures.

Lamfon and Al-Rawi (2007) reported that rats intoxicated with paraquat at a dose level of 1/36 LD50 (3.46 mg/kg BW) three times per week for three weeks induced varying degrees of degenerative changes in the kidneys.

2.6. EFFECTS OF VITAMIN E

Vitamin E (alpha- tocopherol) is a biological anti-oxidant, soluble in fat, which inhibits the oxidation of long chained unsaturated fatty acids of the cell membrane. Unsaturated fatty acid reacts with oxygen, and form superoxide, peroxide and hydroperoxides. These free radicals cause cell damage by disturbing the metabolism and structure of the biological membranes of those organs that contain excessive amount of unsaturated fatty acids. Vitamin E inhibits the effect of hydrogen protons and free radicals by saturating them, and so inhibits autooxidation.

Ehrenkranz *et al.* (1978) studied the effect of vitamin E on the development of brochopulmonary dysplasia in neonates with respiratory-distress syndrome and observed the role of vitamin E in modifying the development of bronchopulmonary dysplasia.

Combs and Peterson (1983) reported that the acute toxicity of paraquat in chick was highly responsive to nutritional selenium status and not vitamin E status.

Pretreatment of mice with 86 units of alpha-tocopherol partially prevented the cardiotoxic effects of alcohol (Redetzki *et al.*, 1983).

Niki *et al.* (1991) found out experimentally that vitamin E, a lipophilic chain-breaking antioxidant present within the membranes, suppresses the oxidative damage of the membranes more efficiently than water-soluble chain-

breaking antioxidants such as vitamin C, which scavenges aqueous radicals but cannot scavenge chain-carrying radicals within the membranes.

A combination of vitamins E and D therapy could be beneficial for the amelioration of fluoride induced changes in reproductive functions in male mice (Chinoy and Sharma, 1998).

Vitamin E may ameliorate organo-phosphate induced oxidative stress by decreasing lipid peroxidation and altering antioxidant defence system in erythrocytes (Susan *et al.*, 2001).

Vitamin E, C and spirulina had a significant antioxidant activity thereby protecting the organs from lead-induced toxicity (Upasani and Balaraman, 2001).

Schwenke *et al.* (2002) observed the protective effects of alpha-tocopherol on diet induced atherosclerosis in NewZealand white rabbits.

Verma and Nair (2002) reported that pretreatment with vitamin E (2mg/animal/day, orally) significantly ameliorated aflatoxin-induced changes in steroidogenesis in mice.

A review of cancer prevention by vitamin E stated that gamma-tocopherol is the most potent form for preventing breast cancer (Kline *et al.*, 2004).

Shekelle *et al.* (2004) studied the effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease and observed that vitamin E supplementation does not beneficially or adversely affect cardiovascular outcomes.

Yonezawa *et al.* (2005) reported the protective effect of Vitamin E against free radicals produced by carbon tetrachloride. Higher activities of aspartate aminotransferase and gamma-glutamyltransferase induced by carbon tetrachloride were reduced by intramuscular injection of vitamin E in steers.

Jalili *et al.* (2007) investigated protective effects of vitamin E on the cardiotoxicity induced by endosulfan in rats.

Vitamin E reduced the detrimental impacts of diazinon as indicated by haematological indices, as well as liver and kidney function in mice. The high dose of diazinon decreased body weight significantly. Moreover, there was a statistically significant decrease in haemoglobin, red blood cells and hematocrit values. Damage in the liver and kidney tissues was also evident as elevated plasma ALT, AST, ALP, urea and creatinine. Vitamin E (100mg/ kg body weight) partially counteracts the toxic effect of diazinon and repairs tissue damage (Nahla *et al.*, 2009).

Raina *et al.* (2009) suggested that alpha-tocopherol supplementation plays a protective role in cypermethrin induced oxidative stress in rats..

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Adult female Sprague Dawley rats weighing approximately 150-200 g procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for the study. Rats were maintained on identical feeding and management practices in the laboratory for one week before the commencement of studies. The experiment was conducted for a period of 28 days.

3.2 SOURCE OF PARAQUAT SAMPLES

The paraquat dichloride (1,1', dimethyl-4, 4'-bipyridylinium dichloride) used was purchased as 24 per cent solution with the trade name Gramaxone , from Syngenta India Ltd, Race course road, Coimbatore, properly sealed in an opaque plastic container. It was kept at room temperature and during use proper caution was taken to avoid spillage, fire or poisoning.

3.3 SOURCE OF VITAMIN E

The vitamin E in the form of vitamin E acetate (DL-alpha-Tocopherol acetate) was purchased from Merck Specialities Private Limited, Worli, Mumbai.

3.4 EXPERIMENTAL DESIGN

Study was conducted in 36 albino rats of either sex weighing 150-200g. They were randomly divided into three groups of 12 each.

Group- I: Served as control group

Group- II: Paraquat dichloride at a dose rate of 25 mg/kg body weight was administered orally three times per week for four weeks using a gastric tube.

Group- III : Paraquat dichloride at a dose rate of 25 mg/kg body weight along with vitamin E at the rate of 200 mg/kg body weight was administered orally three times per week for four weeks.

In the experimental groups, the oral administration was continued up to a period of 28 days and observed for any symptoms. Animals were weighed and blood was collected on day zero and at weekly intervals. Haemoglobin (Hb), Packed Cell Volume (PCV), Erythrocyte sedimentation rate (ESR), Total Leukocyte count (TLC) and Differential Leucocyte count (DLC) were estimated. Serum was used for the estimation of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and creatinine. Six animals each from Group I, II, and III were euthanized at the end of second and forth weeks. Detailed gross examination of all organs was done. Pieces of lung, kidney, liver, heart, stomach, intestine and brain were collected in buffered formalin for histopathology.

Weighed quantity of lungs and kidneys was collected in chilled normal saline for estimation of lipid peroxides and reduced glutathione from all the animals.

3.5 PARAMETERS

3.5.1 **Body Weight**

The body weight of individual rats was recorded at days 0, 7, 14, 21 and 28. From this data, mean body weight was noted. The animals were routinely observed for the clinical signs exhibited.

3.5.2 ***Haematological Parameters***

Blood was collected from the retro-orbital plexus under mild ether anaesthesia with capillary tubes into fresh vials. EDTA was used as the anticoagulant at the rate of 2 mg/ml.

Total Leukocyte count (TLC), Packed Cell Volume (PCV) and Differential Leucocyte count (DLC) were estimated by the method suggested by Thrall *et al.* (2004) on days 0, 7, 14 and 21. Concentration of Haemoglobin (Hb) was estimated by acid haematin method and ESR was estimated as per standard procedure (Feldman *et al.*, 2000).

3.5.3 **Biochemical Studies**

Blood was collected from the retro-orbital plexus under mild ether anaesthesia with capillary tubes, into clean vials (non-heparinised) and allowed to clot. The serum was separated from the clot and then it was centrifuged at 2000 rpm for 20 minutes. The clear serum was aspirated into another vial and used for biochemical analysis. Estimation of serum ALT, AST, ALP and creatinine was done using kits manufactured by Agappe diagnostics.

3.5.4 **Lipid Peroxides and Reduced Glutathione**

The animals were sacrificed and the lungs and kidneys were collected. Tissues were washed in running tap water to remove the blood clots and weighed amount of tissue was kept in chilled normal saline.

3.5.4.1 ***Estimation of Tissue Reduced Glutathione***

Levels of reduced Glutathione in lung and kidney homogenate were estimated by the method adapted by Moron *et al.* (1979).

a. Principle

Reduced glutathione was measured by its reaction with 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) to give a yellow coloured complex with absorption maximum at 412 nm.

b. Reagents

1. Phosphate buffer – 0.2 mol, pH 8.0
2. Trichloroacetic acid (TCA)- 5 per cent
3. Trichloroacetic acid (TCA)- 25 per cent
4. DTNB – 0.6 mMol.

c. Procedure

1. Preparation of tissue homogenate:

   Homogenate of lung and kidney were prepared in the ratio of 0.5g of wet tissue to 4 ml of phosphate buffer. It was then centrifuged at 5000rpm for 5 minutes and the supernatant was used for the estimation of reduced glutathione.

2. 125 µl of 25 per cent Trichloroacetic acid was added to 500 µl of supernatant from the tissue homogenate taken in a test tube, for the precipitation of proteins and mixed well.

3. The tubes were then cooled in ice bath for 5 minutes.

4. The mixture was again diluted with 575 µl of 5 per cent TCA and centrifuged for 5 minutes at 5000 rpm.

5. 300 µl of the supernatant was transferred into another test tube and 700 µl of phosphate buffer was added to it.

6. To the above mixture, 2 ml of freshly prepared DTNB was added, mixed well and the yellow color formed was read at 412 nm using genesis spectrophotometer.

d. Preparation of standard curve

Standard curve of glutathione was prepared by using concentrations varying from 1-10 µg of glutathione standard which was dissolved in 5 per cent TCA. The volume of standard solution was made up to 1 ml with 0.2 mol phosphate buffer. Added 2 ml of freshly prepared 0.6 mMol DTNB to the tubes and the intensity of yellow color formed was read at 412 nm. A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of the reduced glutathione was read directly from the calibration curve and expressed as µg/g wet tissue.

3.5.4.2 ***Estimation of Lipid Peroxides***

The levels of lipid peroxides in lung and kidney tissues were estimated by the method of Ohkawa *et al.* (1979).

a. Principle:

Thiobarbituric acid (TBA) reacts with malondialdehyde, an end product of fatty acid peroxidation to form a red colored pigment, which has maximum absorbance at 532 nm. 1, 1, 3, 3 tetra methoxy propane was used as standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

b. Reagents:

1. Potassium chloride – 1.15 per cent

2. Sodium dodecyl sulphate – 8.1 per cent (SDS)

3. Acetic acid - 20 per cent; PH adjusted to 3.5 with sodium Hydroxide

4. Thiobarbituric acid (TBA) - 0.8 per cent in hot distilled water

5. n-butanol

6. Standard solution - 1,1,3,3 tetra methoxy propane (4.8 nMol).

c. Procedure:

1. One gram of tissue was mixed with 9ml of 1.15 per cent potassium chloride and homogenized in a tissue homogenizer.
2. Centrifuged the homogenate at 5000 rpm for 5 minutes.
3. Supernatant (0.1 ml) was taken in test tube, added 0.2 ml of 8.1 per cent SDS, 1.5 ml of 20 per cent acetic acid and 1.5 ml of 0.8 per cent TBA.
4. Made up the volume to 4 ml with distilled water and heated in a water bath at 95^0 C for 60 minutes.
5. Cooled under tap water.
6. Added 1 ml of distilled water and 5 ml of n- butanol and shaken vigorously
7. Centrifuged at 4000 rpm for 10 minutes.
8. The absorbance of the colour of the organic layer was measured at 532 nm using n- butanol as blank.

d. Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 nMol to 5 nMol of 1,1,3,3 tetra methoxy propane in double distilled water by following the above procedure. A graph was plotted between optical density and

concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nMol of malondialdehyde/g of wet tissue.

3.6 PATHOANATOMICAL STUDIES

Six animals each from Group I, II, and III were euthanized at the end of second and forth weeks. Detailed postmortem examination was conducted and gross lesions were recorded. Pieces of lung, kidney, liver, heart, stomach, intestine and brain were collected in buffered formalin for histopathology. They were processed and embedded in paraffin and sections were cut at 5 μ thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Cook, 1995).

3.7 STATISTICAL ANALYSIS

Data collected from various parameters were analysed as per the method of Snedecor and Cochran (1994) by using one way analysis of variance (ANOVA) and followed by Duncans multiple range test for grouping means having significance.

Results

4. RESULTS

The experiment was conducted to study the pathology of paraquat toxicity in rats and to evaluate the ameliorative effect of vitamin E in paraquat toxicity. The results are analysed and presented in tables and figures in this chapter.

4.1 PHYSIOLOGICAL PARAMETERS

4.1.1 **Body Weight**

The mean body weights of rats of Group I, II, and III were recorded on day 0, 7, 14, 21 and 28 of the experiment and are presented in the table1. Body weight of rats of all groups showed a gradual increase throughout the experimental period. The mean body weight of treatment groups did not differ significantly with that of control group ($p>0.05$).

4.2 BIOCHEMICAL PARAMETERS

4.2.1 **Aspartate amino transferase (AST)**

The mean AST levels of group I, II and III on day 0, 7, 14, 21 and 28 are shown in Table 2 and Fig.1. A significant increase ($p<0.01$) in the AST levels was observed between treatment groups on day 14, 21 and 28 when compared to the control group. The mean AST levels of group I, II and III on day 0 and 28 were 150.45 ± 7.84 IU/L, 146.12 ± 10.75 IU/L, 153.69 ± 11.98 IU/L and 150.69 ± 6.74 IU/L, 218.23 ± 7.66 IU/L, 217.42 ± 14.09 IU/L respectively. A slight non significant decrease in AST values was noticed in vitamin E treated group compared to control group.

4.2.2 **Alanine amino transferase (ALT)**

The mean ALT levels of group I, II and III on day 0, 7, 14, 21 and 28 are shown in Table 3 and Fig. 2. A significant increase ($p<0.01$) in the ALT levels

was observed in treatment groups on day 7, 14, 21 and 28 when compared to the control group. A gradual increase in the ALT levels was noticed in both group II and III. Treatment with vitamin E did not result in significant decrease in the level of serum ALT in group III as compared to group II animals.

4.2.3 **Alkaline phosphatase (ALP)**

The mean values of serum alkaline phosphatase level on day 0, 7, 14, 21 and 28 are listed in Table 4 and Fig. 3. The mean ALP levels of treatment groups differ significantly with that of control group ($p<0.05$) on day 7, 14, 21 and 28. Though statistically not significant, when compared to the group treated with paraquat alone, vitamin E treated group showed a slight decrease in ALP values.

4.2.4 **Creatinine**

The results obtained are presented in the Table 5 and Fig. 4. The mean creatinine value showed significant increase ($P<0.01$) on day 7, 14, 21 and 28 day when compared to control group. No statistically significant difference was noticed between group II and III.

4.3 HAEMATOLOGICAL PARAMETERS

4.3.1 **Erythrocyte sedimentation rate (ESR)**

The mean ESR values of all experimental groups showed no significant difference when compared with the control group (Table 6).

4.3.2 **Haemoglobin (Hb)**

The mean haemoglobin values of experimental groups showed significant decrease when compared with the control group on day 7, 14, 21 and 28. But the experimental groups in between did not differ significantly (Table 7 and Fig. 5).

4.3.2 **Packed Cell Volume (PCV)**

The results are shown in Table 8 and Fig. 6. The PCV values in the experimental groups were significantly decreased when compared with the control group on day 7, 14, 21 and 28. But the mean PCV values of rats in group II were statistically comparable with those in group III.

4.3.4 **Total Leukocyte Count (TLC)**

The mean TLC values are listed in the table 9 and Fig.7. Results indicated that there was no variations in the TLC value between the groups on day 0, 7, 14 and 21. On day 28, group II and III showed significant increase in TLC values when compared with the control group. On day 28, the mean TLC values of group I, II, and III were 7.29 ± 1.22, 8.04 ± 1.45 and 7.66 ± 1.625 thousands per mm^3 respectively. Treatment with vitamin E caused only a slight decrease in TLC values on day 28 in group III when compared with group II.

4.3.5 **Differential leukocyte Count (DLC)**

Table 10 represents the mean DLC values. All the values of DLC were within the normal range.

4.4 CLINICAL SIGNS AND MORTALITY PATTERN

None of the animals in the experimental groups revealed any signs of toxicity for the first three weeks of experiment. On 25^{th} day of experiment one of the rats from group II became dull. One rat from group III also became weak and anorectic from 27^{th} day onwards. All other animals were found to be apparently normal and active throughout the experiment.

4.5 OXIDATIVE EFFECT ON LUNGS AND KIDNEYS

4.5.1 **Lipid Peroxides**

The mean values of lipid peroxides in the lungs of rats sacrificed on 14th and 28th day of treatment are presented in Table 11 and Figure 8. Among the animals sacrificed on 14th day of treatment, Group II (143.83 ± 5.91nMol/g) and III (137.67± 9.86 nMol/g) revealed significant increase ($P<0.01$) in the mean values of lipid peroxides in lungs when compared with control group (106.00 ± 11.80 nMol/g). The animals in group II and III sacrificed at the end of experiment showed further increase ($P<0.01$) in the mean values of lipid peroxides when compared to rats sacrificed on 14th day of treatment.

In the kidneys also, group II and III showed significant increase ($P<0.01$) when compared with control group at one per cent level (Table 12 and Figure 9). But the group II and group III did not differ significantly in mean values of lipid peroxides in both lungs and kidneys.

4.5.2 **Reduced Glutathione**

The mean values of reduced glutathione in lungs of rats sacrificed on 14th and 28thday of treatment are presented in Table 13 and Figure10. The animals of Group II and III showed significant decrease in the mean values of reduced glutathione in lungs at one per cent level when compared with control.

The mean values of reduced glutathione in kidneys of rats sacrificed on 14th and 28thday of treatment are presented in Table 14 and Figure11. Group II and III revealed significant decrease ($P<0.01$) in the mean values of reduced glutathione in kidneys when compared with control group. Treatment with vitamin E did not cause significant increase in the level of reduced glutathione in lungs and kidneys of group III when compared with group II animals.

4.6 PATHOANATOMICAL STUDIES

Six animals each from Group I, II, and III were sacrificed at the end of second and fourth weeks. Detailed gross and histopathological examination of all organs was done.

4.6.1 **Gross lesions**

Gross lesions were seen predominantly in the lungs of rats. Moderate to marked degree of congestion with varying degrees of emphysema was noticed in the lungs of Group II and III animals when compared to control (Fig. 12 & 13). Kidneys and liver showed moderate degree of congestion in both Group II and III (Fig. 14, 15 & 16). Gastric mucosa was found to be slightly hyperemic in Group II animals (Fig. 17). All other organs were found to be apparently normal. Gross lesions were almost similar in animals sacrificed at the end of second and forth weeks.

4.6.2 HISTOPATHOLOGICAL LESIONS

4.6.2.1 **Group I- Control group**

The lungs, kidneys, liver, heart, brain, stomach and intestine did not show any histopathological lesions.

4.6.2.2 ***Group II***

4.6.2.2.1 ***Group II animals sacrificed at the end of second week***

The rats in group II were administered with paraquat alone and based on the lesions, the affected organs were mainly lungs, kidneys and the liver.

Lungs

The lungs showed congestion of the blood vessels and focal areas of emphysema. Mild to moderate degree of thickening of the interalveolar septa was observed due to congestion and mild degree of metaplastic changes of the alveolar epithelium to fibroblast cells. Desquamated and proliferated epithelium was seen in the lumen of the bronchi mixed with RBCs (Fig. 18). Alveolar epithelium was found to be proliferated. Mild depletion of the lymphatic tissue associated with bronchi and proliferation of fibroblasts in the peribronchial area were also observed. In a few animals, lung parenchyma also revealed focal areas of necrosis with infiltration of mononuclear type of inflammatory cells and proliferating fibroblast type of cells arranged in whorls (Fig. 19 & 20).

Kidneys

In the kidneys, focal areas of haemorrhage were noticed in the intertubular spaces (Fig. 21). The tubular epithelium was swollen and there was narrowing of the lumen (Fig. 22). The epithelial cells also showed erosion of the free cellular borders. Few glomeruli also had degenerative changes. Bowman's capsule was found to be intact.

Liver

The central and portal veins were severely congested (Fig. 23). Sinusoidal congestion and dilatation was also noticed. Most of the hepatocytes revealed granular cytoplasm (Fig. 24). Moderate proliferation of bile duct epithelium was also observed.

Other lesions

In the heart, thinning and separation of cardiac muscle fibres and mild degree of fatty change were observed (Fig. 25). Mild congestion of the blood vessels and focal areas of haemorrhage in between cardiac muscle fibres were

also noticed (Fig. 26). Mild degenerative changes of the glandular epithelium were noticed in the stomach. Microscopic examination of intestine revealed mild degree of goblet cell hyperplasia and necrotic changes affecting the tips of the villi (Fig. 27). Necrosis of the mucosal epithelium was also observed. Mild oedema evidenced by enlargement of perivascular space was noticed in the brain (Fig. 28).

4.6.2.2.2 ***Group II animals sacrificed at the end of forth week***

Lungs

Thickening of the interalveolar septa due to proliferation of the fibroblast cells were prominent (Fig. 29). The number of the intact alveoli was decreased and some of the alveoli were filled with edematous fluid containing erythrocytes and fibroblast-like cells. Congestion of the blood vessels and diffuse hemorrhage in the interstitium were also observed. Alveolar epithelium revealed varying degree of degenerative to necrotic changes. Focal areas of emphysema were also present. The bronchial epithelium revealed proliferative changes and desquamation along with hyalinization of the epithelial surface (Fig. 30).

Kidneys

Kidney revealed congestion, severe degeneration and necrosis of tubular epithelium when compared with animals euthanized at the end of second week (Fig. 31).

Liver

Blood vessels were severely congested. In the liver multifocal necrosis and sinusoidal congestion were observed. Most of the hepatocytes revealed pyknotic nuclei and vacoular cytoplasm (Fig. 32).

Other lesions

Histopathological examination of heart revealed mild degree of fatty change and haemorrhage between cardiac muscle fibres (Fig.33). Stomach revealed degenerative changes. In the intestine goblet cell hyperplasia, mild depletion of Peyer's patches and necrosis of the tip of the villi were noticed (Fig. 34). Brain sections showed severe oedema affecting both white matter and grey matter.

4.6.2.3 **Group III**

4.6.2.3.1 ***Group III animals sacrificed at the end of second week***

Lungs

Lungs revealed congestive changes. Bronchi associated lymphoid nodules were intact and active. Bronchial epithelium was intact with very little bronchial exudate. Proliferation of fibroblasts though present were less (Fig. 35).

Kidneys

Renal tubular epithelium was swollen and projected into the lumen. Focal areas of haemorrage were noticed in the intertubular spaces. Focal tubular necrotic changes were also observed (Fig. 36).

Liver

Liver showed slight degenerative changes, mild fatty change, sinusoidal dilatation and Kupffer cell proliferation when compared with Group II (Fig. 37). The congestive changes were less severe in this group.

Other lesions

Heart revealed densely packed muscle fibres with mild degree of congestion and haemorrhage (Fig. 38). Cardiac muscle fibres also showed slight fatty change. Intestine showed mild goblet cell hyperplasia and focal necrosis of the tips of the villi (Fig. 39). The lymphoid aggregates were found to be intact. No appreciable changes were noticed in the stomach and brain (Fig. 40).

4.6.2.3.2 ***Group III animals sacrificed at the end of forth week***

Lungs

Blood vessels were congested. Bronchial epithelium was intact when compared with group II animals. Alveolar septa were almost normal except for mild proliferation of fibroblasts.

Kidneys

Mild degenerative changes were present in the renal tubular epithelium. Mild congestive changes were also noticed (Fig. 41). Glomeruli were found to be apparently normal.

Liver

Liver revealed degeneratve changes of hepatocytes. Focal necrotic changes were also noticed (Fig. 42). The central venous congestion and sinusoidal dilatation were less severe when compared to group II.

Other lesions

Heart revealed densely packed muscle fibres. Histopathological examination of intestine revealed mild goblet cell hyperplasia. Sections of brain did not show any lesions.

Table 1: Effect of paraquat dichloride and vitamin E on mean body weight (g) of rats

Days	Group I	Group II	Group III
0	$150^{a} \pm 2.13$	$144.17^{a} \pm 2.29$	$150^{a} \pm 2.13$
7	$153.33^{a} \pm 2.56$	$148.33^{a} \pm 2.07$	$152.50^{a} \pm 2.79$
14	$167.50^{a} \pm 2.50$	$160.33^{a} \pm 1.88$	$165^{a} \pm 2.61$
21	$171.67^{a} \pm 6.01$	$163.33^{a} \pm 2.11$	$173.33^{a} \pm 4.94$
28	$185^{a} \pm 7.64$	$168.33^{a} \pm 7.03$	$178.33^{a} \pm 6.01$

(Means bearing same superscript in the same row does not differ significantly)

Table 2: Effect of paraquat dichloride and vitamin E on mean values of aspartate aminotransferase (IU/L) in rats

Days	Group I	Group II	Group III
0	$150.45^{a} \pm 7.84$	$146.12^{a} \pm 10.75$	$153.69^{a} \pm 11.98$
7	$158.16^{a} \pm 6.67$	$174.86^{a} \pm 4.16$	$163.29^{a} \pm 9.13$
14	$148.23^{a} \pm 6.11$	$187.23^{b} \pm 4.31$	$167.78^{ab} \pm 11.36$
21	$148.23^{a} \pm 6.09$	$207.05^{b} \pm 4.24$	$209.57^{b} \pm 15.22$
28	$150.69^{a} \pm 6.74$	$218.23^{b} \pm 7.66$	$217.42^{b} \pm 14.09$

(Means bearing same superscript in the same row does not differ significantly)

Table 3: Effect of paraquat dichloride and vitamin E on mean values of alanine aminotransferase (IU/L) in rats

Days	Group I	Group II	Group III
0	$40.87^{a} \pm 4.06$	$43.29^{a} \pm 4.03$	$37.39^{a} \pm 3.02$
7	$44.51^{a} \pm 4.56$	$73.86^{b} \pm 5.71$	$66.33^{b} \pm 4.26$
14	$45.11^{a} \pm 2.95$	$87.99^{b} \pm 6.80$	$83.19^{b} \pm 4.00$
21	$48.74^{a} \pm 5.02$	$119.29^{b} \pm 8.03$	$106.00^{b} \pm 11.81$
28	$46.21^{a} \pm 6.74$	$128.88^{b} \pm 7.66$	$122.13^{b} \pm 14.09$

(Means bearing same superscript in the same row does not differ significantly)

Table 4: Effect of paraquat dichloride and vitamin E on mean values of alkaline phosphatase (IU/L) in rats

Days	Group I	Group II	Group III
0	$127.88^{a} \pm 10.04$	$128.62^{a} \pm 14.04$	$123.32^{a} \pm 19.84$
7	$126.18^{a} \pm 10.63$	$177.15^{b} \pm 9.72$	$166.66^{ab} \pm 19.65$
14	$139.42^{a} \pm 14.89$	$229.38^{b} \pm 11.66$	$231.29^{b} \pm 17.59$
21	$125.88^{a} \pm 21.07$	$342.08^{b} \pm 16.40$	$341.55^{b} \pm 21.69$
28	$136.13^{a} \pm 10.46$	$412.25^{b} \pm 16.06$	$389.97^{b} \pm 18.08$

(Means bearing same superscript in the same row does not differ significantly)

Table 5: Effect of paraquat dichloride and vitamin E on mean values of creatinine (mg/dl) in rats

Days	Group I	Group II	Group III
0	$0.272^{a} \pm 0.036$	$0.319^{a} \pm 0.021$	$0.297^{a} \pm 0.041$
7	$0.368^{a} \pm 0.026$	$0.449^{b} \pm 0.018$	$0.404^{b} \pm 0.039$
14	$0.378^{a} \pm 0.035$	$0.506^{b} \pm 0.019$	$0.428^{ab} \pm 0.025$
21	$0.385^{a} \pm 0.038$	$0.603^{b} \pm 0.011$	$0.552^{b} \pm 0.024$
28	$0.383^{a} \pm 0.012$	$0.632^{b} \pm 0.019$	$0.608^{b} \pm 0.024$

(Means bearing same superscript in the same row does not differ significantly)

Table 6: Effect of paraquat dichloride and vitamin E on mean erythrocyte sedimentation rate (mm/hr) of rats

Days	Group I	Group II	Group III
0	$2.71^{a} \pm 0.20$	$2.58^{a} \pm 0.19$	$3.08^{a} \pm 0.29$
7	$2.75^{a} \pm 0.18$	$2.67^{a} \pm 0.22$	$2.67^{a} \pm 0.19$
14	$2.92^{a} \pm 0.19$	$2.83^{a} \pm 0.21$	$3.58^{a} \pm 0.31$
21	$2.67^{a} \pm 0.21$	$3.17^{a} \pm 0.31$	$3.00^{a} \pm 0.37$
28	$2.83^{a} \pm 0.31$	$3.17^{a} \pm 0.31$	$3.17^{a} \pm 0.31$

(Means bearing same superscript in the same row does not differ significantly)

Table 7: Effect of paraquat dichloride and vitamin E on mean haemoglobin concentration (g/dl) of rats

Days	Group I	Group II	Group III
0	$16.32^{a} \pm 0.44$	$14.59^{a} \pm 0.43$	$15.18^{a} \pm 0.38$
7	$16.49^{b} \pm 0.40$	$13.74^{a} \pm 0.48$	$14.35^{a} \pm 0.28$
14	$16.28^{b} \pm 0.38$	$13.11^{a} \pm 0.43$	$14.07^{a} \pm 0.39$
21	$15.75^{b} \pm 0.60$	$12.78^{a} \pm 1.05$	$13.97^{a} \pm 0.74$
28	$15.42^{b} \pm 0.41$	$12.11^{a} \pm 0.81$	$13.23^{a} \pm 0.76$

(Means bearing same superscript in the same row does not differ significantly)

Table 8: Effect of paraquat dichloride and vitamin E on mean packed cell volume (%) of rats

Days	Group I	Group II	Group III
0	$41.33^{a} \pm 0.85$	$40.63^{a} \pm 0.91$	$40.74^{a} \pm 0.77$
7	$41.33^{b} \pm 0.74$	$38.35^{a} \pm 0.86$	$38.19^{a} \pm 0.59$
14	$41.15^{b} \pm 0.50$	$34.99^{a} \pm 0.77$	$36.18^{a} \pm 0.72$
21	$40.25^{b} \pm 0.68$	$33.60^{a} \pm 1.11$	$35.47^{a} \pm 1.10$
28	$41.02^{b} \pm 0.74$	$32.25^{a} \pm 0.99$	$34.72^{a} \pm 0.93$

(Means bearing same superscript in the same row does not differ significantly)

Table 9: Effect of paraquat dichloride and vitamin E on mean total leukocyte count (thousands per mm^3) of rats

Days	Group I	Group II	Group III
0	$7.02^a \pm 1.7586$	$7.37^a \pm 1.97$	$6.93^a \pm 1.836$
7	$7.10^a \pm 1.90$	$7.46^a \pm 1.30$	$7.48^a \pm 1.07$
14	$7.12^a \pm 1.713$	$7.62^a \pm 1.456$	$7.43^a \pm 1.51$
21	$7.28^a \pm 1.657$	$7.84^a \pm 0.80$	$7.53^a \pm 1.923$
28	$7.29^a \pm 1.22$	$8.04^b \pm 1.45$	$7.66^{ab} \pm 1.625$

(Means bearing same superscript in the same row does not differ significantly)

Table 10: Effect of paraquat dichloride and vitamin E on mean differential leukocyte count (%) of rats

Days		Group I	Group II	Group III
0	Lymphocyte	$79.25^a \pm 0.83$	$79.75^a \pm 0.86$	$79.17^a \pm 1.11$
	Neutrophil	$15.75^a \pm 0.83$	$16.25^a \pm 0.86$	$15.83^a \pm 1.11$
	Eosinophil	$2.25^a \pm 0.11$	$1.98^a \pm 0.23$	$2.30^a \pm 0.22$
	Monocyte	$3.45^a \pm 0.09$	$2.85^a \pm 0.21$	$3.23^a \pm 0.26$
7	Lymphocyte	$80.25^a \pm 1.07$	$78.58^a \pm 1.22$	$77.17^a \pm 1.36$
	Neutrophil	$15.75^a \pm 1.07$	$16.42^a \pm 1.22$	$17.83^a \pm 1.36$
	Eosinophil	$2.31^a \pm 0.31$	$2.41^a \pm 0.24$	$1.88^a \pm 0.30$
	Monocyte	$3.34^a \pm 0.26$	$2.76^a \pm 0.31$	$3.12^a \pm 0.25$
14	Lymphocyte	$80.25^a \pm 1.37$	$80.17^a \pm 0.93$	$78.17^a \pm 1.22$
	Neutrophil	$13.92^a \pm 1.22$	$15.83^a \pm 0.93$	$18.83^a \pm 1.22$
	Eosinophil	$2.23^a \pm 0.12$	$1.89^a \pm 0.56$	$2.13^a \pm 0.25$
	Monocyte	$3.19^a \pm 0.25$	$2.65^a \pm 0.34$	$1.08^a \pm 0.51$

Table 10 Continued

Days		Group I	Group II	Group I
21	Lymphocyte	$79.33^{a} \pm 1.84$	$79.00^{a} \pm 1.24$	$78.17^{a} \pm 1.19$
	Neutrophil	$15.67^{a} \pm 1.84$	$16.00^{a} \pm 1.24$	$18.00^{a} \pm 1.13$
	Eosinophil	$2.35^{a} \pm 0.22$	$2.33^{a} \pm 0.10$	$2.56^{a} \pm 0.24$
	Monocyte	$2.51^{a} \pm 0.22$	$3.12^{a} \pm 0.11$	$1.75^{a} \pm 0.31$
28	Lymphocyte	$80.00^{a} \pm 1.24$	$79.83^{a} \pm 1.01$	$79.00^{a} \pm 1.13$
	Neutrophil	$16.00^{a} \pm 1.24$	$15.17^{a} \pm 1.01$	$16.00^{a} \pm 1.13$
	Eosinophil	$1.89^{a} \pm 0.42$	$2.45^{a} \pm 0.53$	$2.14^{a} \pm 0.27$
	Monocyte	$2.45^{a} \pm 0.39$	$2.34^{a} \pm 0.45$	$2.67^{a} \pm 0.26$

(Means bearing same superscript in the same row does not differ significantly)

Table 11: Effect of paraquat dichloride and vitamin E on mean values of lipid peroxide (nMol/g) in lungs of rats sacrificed on 14^{th} and 28^{th} day of treatment

Group	Day 14	Day 28
Group I	$106.00^{a} \pm 11.80$	$110.17^{a} \pm 7.64$
Group II	$143.83^{b} \pm 5.91$	$216.83^{b} \pm 9.73$
Group III	$137.67^{b} \pm 9.86$	$214.83^{b} \pm 11.48$

(Means bearing same superscript in the same column does not differ significantly)

Table 12: Effect of paraquat dichloride and vitamin E on mean values of lipid peroxide (nMol/g) in kidneys of rats sacrificed on 14th and 28th day of treatment

Group	Day 14	Day 28
Group I	$186.67^{a} \pm 9.15$	$198.17^{a} \pm 8.43$
Group II	$254.67^{b} \pm 10.19$	$315.50^{b} \pm 14.39$
Group III	$257.17^{b} \pm 14.51$	$299.83^{b} \pm 13.95$

(Means bearing same superscript in the same column does not differ significantly)

Table 13: Effect of paraquat dichloride and vitamin E on mean values of reduced glutathione (µg /g) in lungs of rats sacrificed on 14th and 28th day of treatment

Group	Day 14	Day 28
Group I	$391.50^{b} \pm 4.69$	$394.50^{b} \pm 7.63$
Group II	$149.67^{a} \pm 4.63$	$137.67^{a} \pm 4.70$
Group III	$153.67^{a} \pm 5.15$	$156.67^{a} \pm 4.14$

(Means bearing same superscript in the same column does not differ significantly)

Table 14: Effect of paraquat dichloride and vitamin E on mean values of reduced glutathione (µg/g) in kidneys of rats sacrificed on 14th and 28th day of treatment

Group	Day 14	Day 28
Group I	$607.00^{b} \pm 8.44$	$601.50^{b} \pm 8.83$
Group II	$361.00^{a} \pm 6.04$	$323.00^{a} \pm 9.98$
Group III	$380.00^{a} \pm 8.05$	$333.17^{a} \pm 10.30$

(Means bearing same superscript in the same column does not differ significantly)

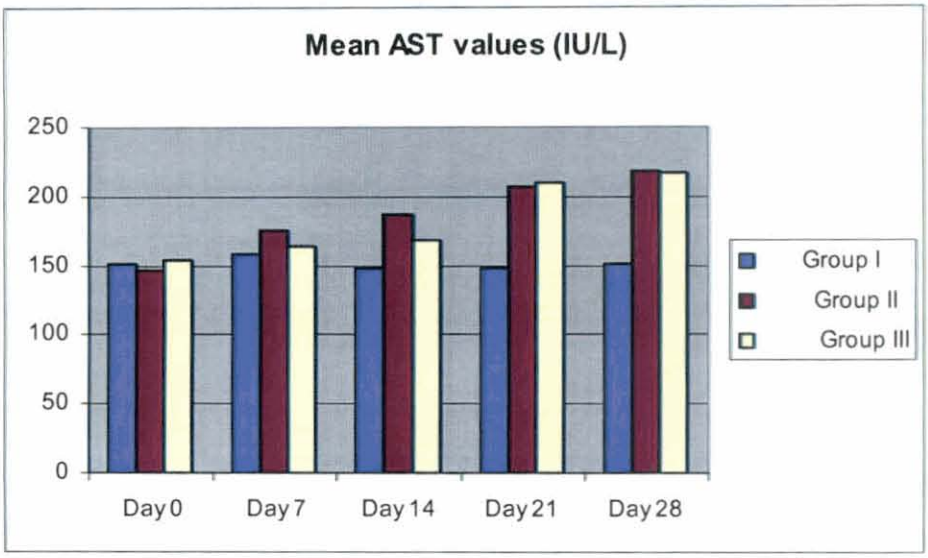

Fig.1 Mean AST values (IU/L)

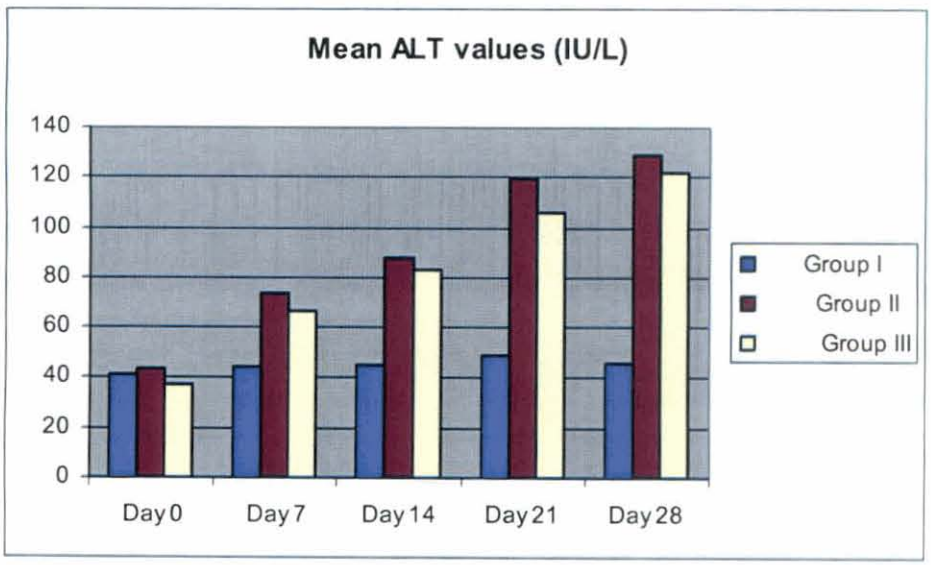

Fig.2 Mean ALT values (IU/L)

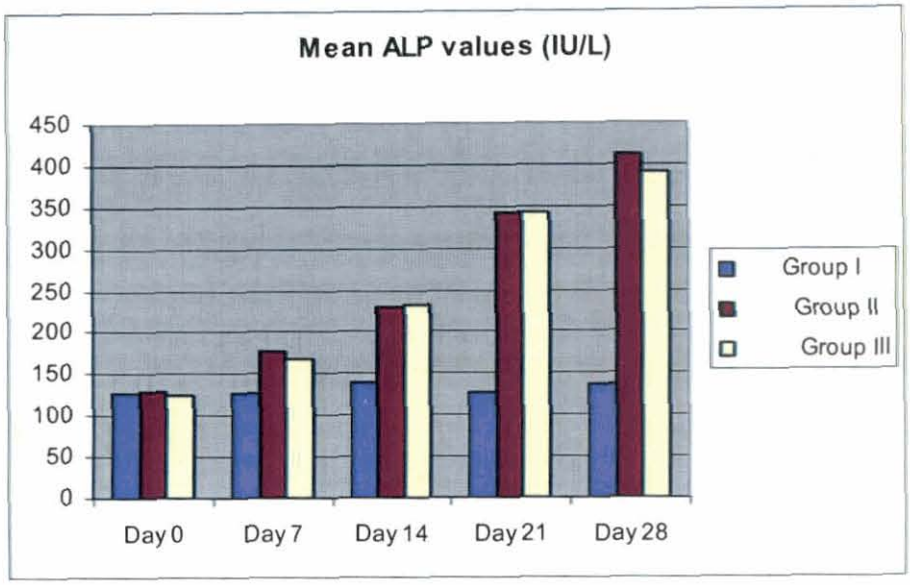

Fig. 3 Mean ALP values (IU/L)

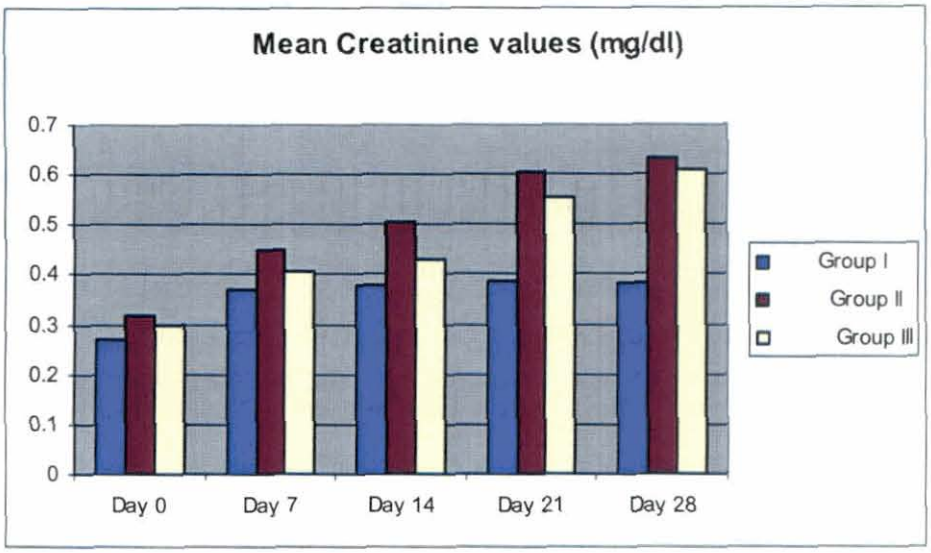

Fig.4 Mean Creatinine values (mg/dl)

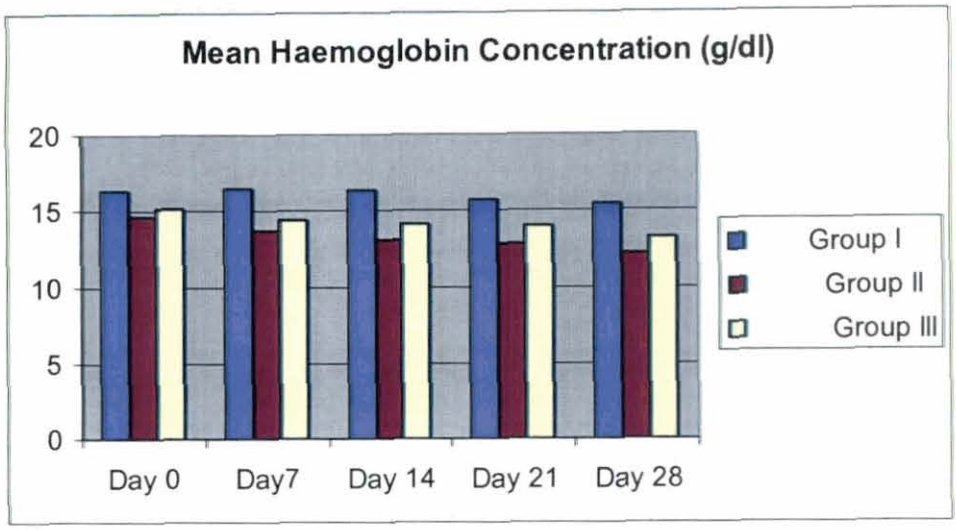

Fig.5 Mean Haemoglobin Concentration (g/dl)

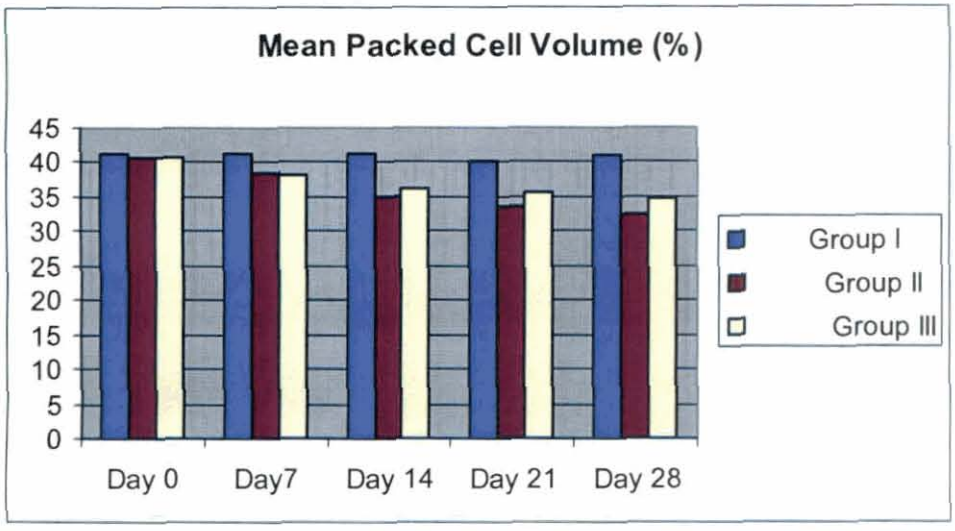

Fig.6 Mean Packed Cell Volume (%)

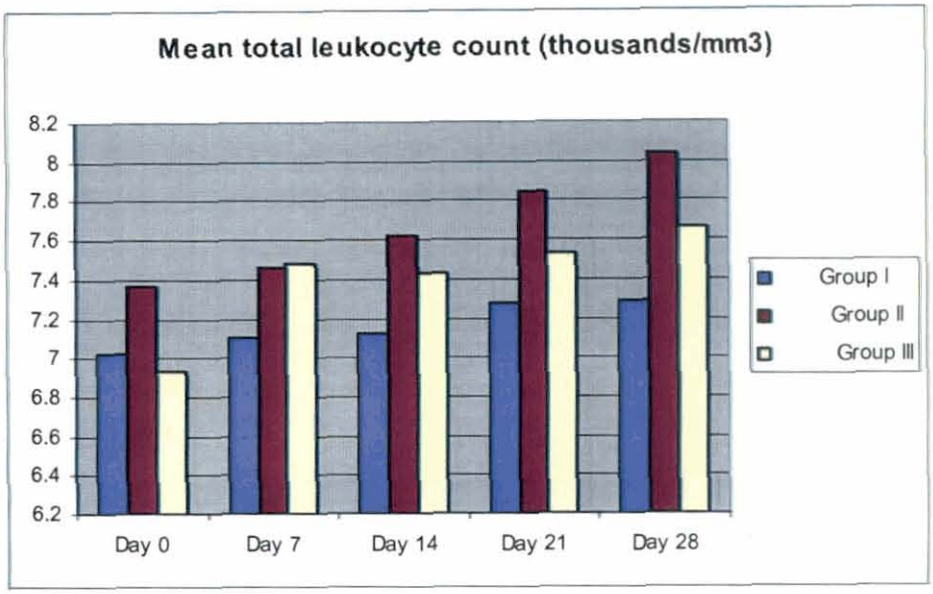

Fig.7 Mean total leukocyte count (thousands per mm^3)

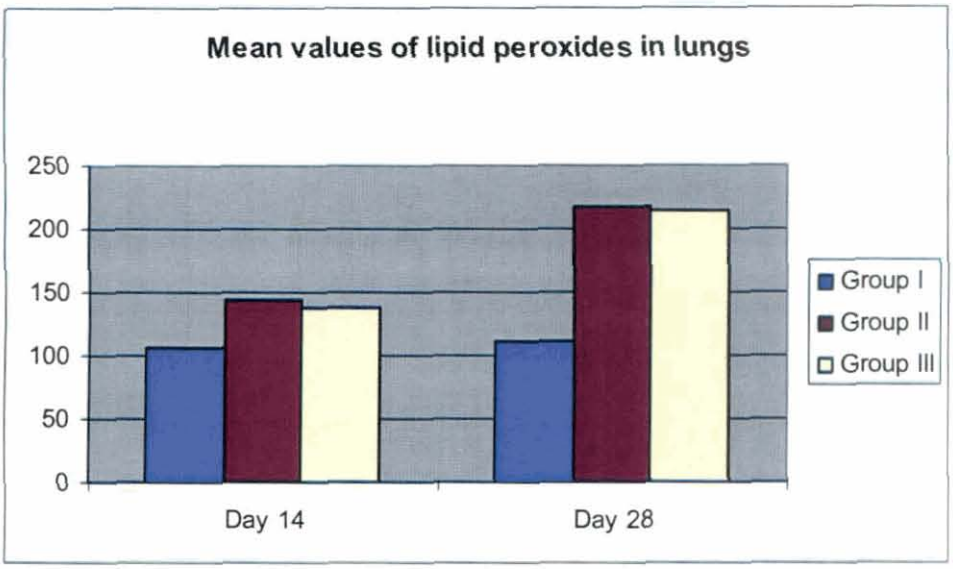

Fig.8 Mean values of lipid peroxides in lungs (μg/g)

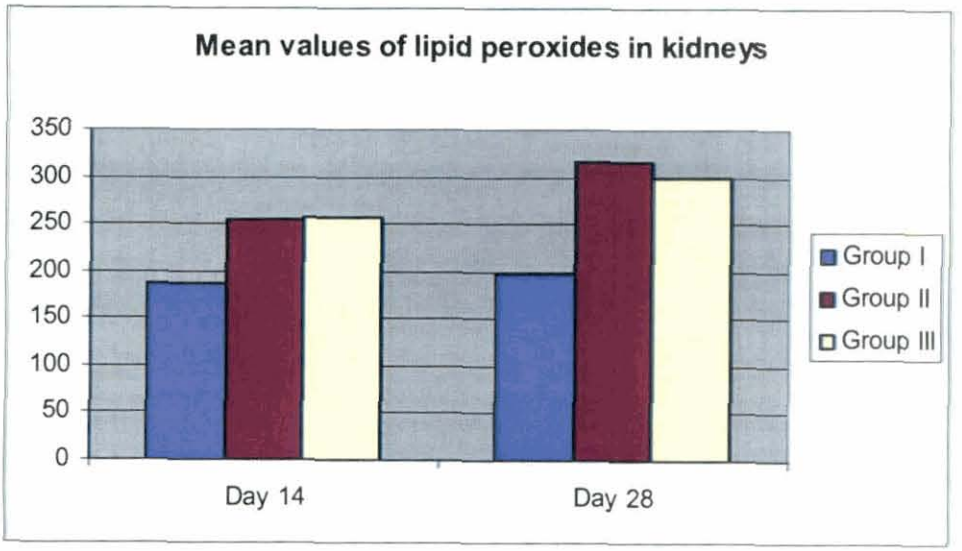

Fig.9 Mean values of lipid peroxides in kidneys (μg/g)

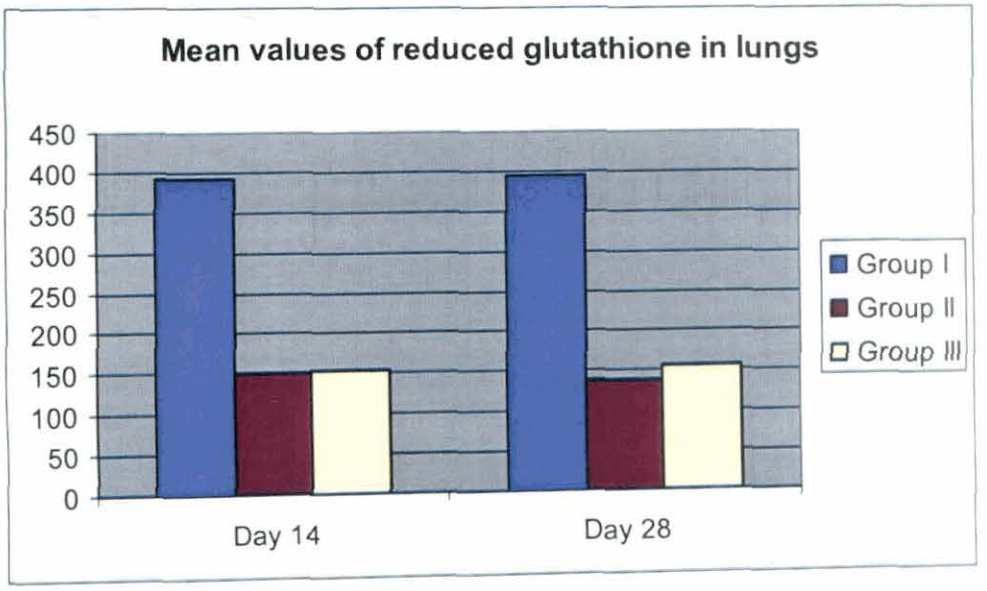

Fig.10 Mean values of reduced glutathione in lungs (nMol /g)

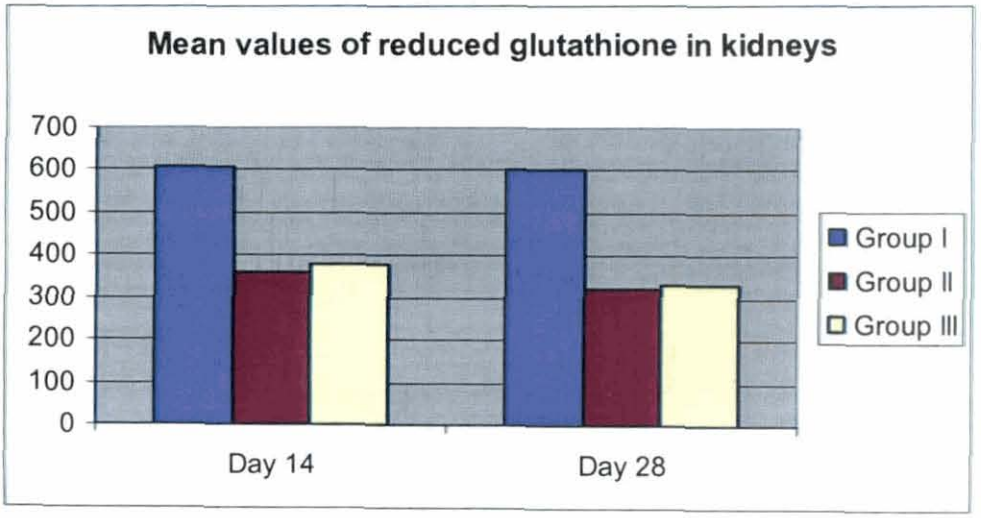

Fig.11 Mean values of reduced glutathione in kidneys (nMol /g)

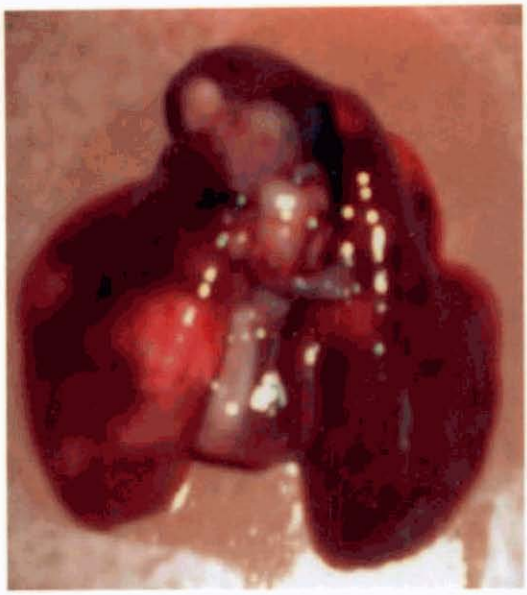

Fig. 12

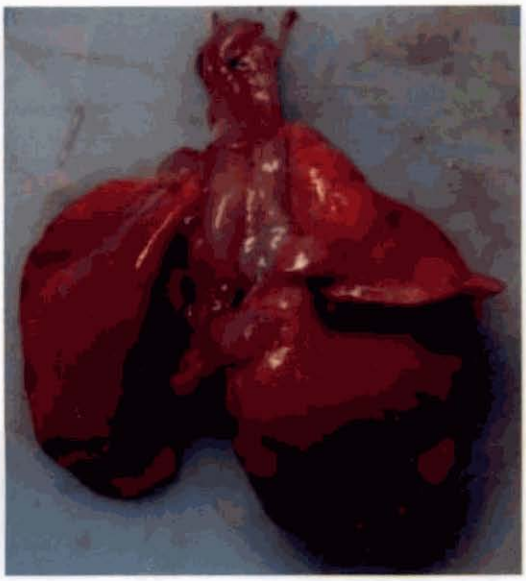

Fig. 13

Fig. 12 Lungs - Marked congestion and Emphysema - Group II

Fig. 13 Lungs – Moderate congestion and Emphysema - Group III

Group II : Treated with paraquat dichloride alone (25 mg/kg body weight)

Group III : Treated with paraquat dichloride (25 mg/kg body weight) along with vitamin E (200 mg/kg body weight)

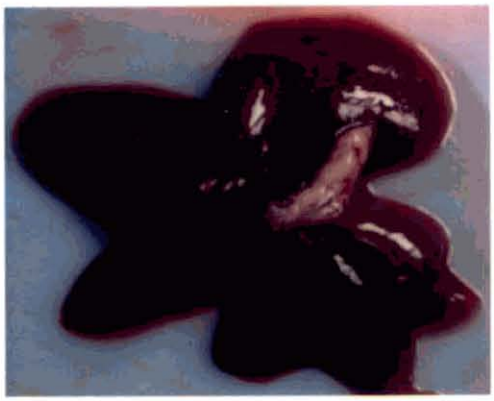

Fig.14

Fig.15

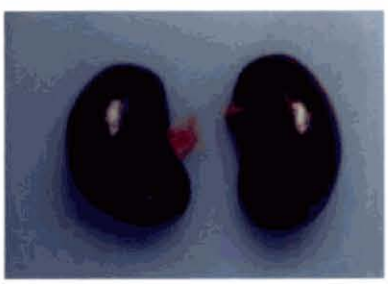

Fig. 16

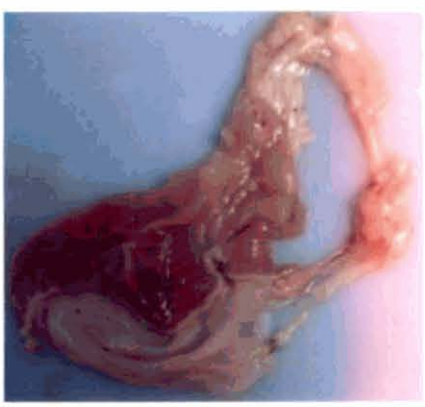

Fig. 17

Fig. 14 Liver - Marked congestion – Group II

Fig. 15 Liver - Moderate congestion – Group III

Fig. 16 Kidney - Congestion – Group II

Fig. 17 Stomach - Hyperemic mucosa – Group II

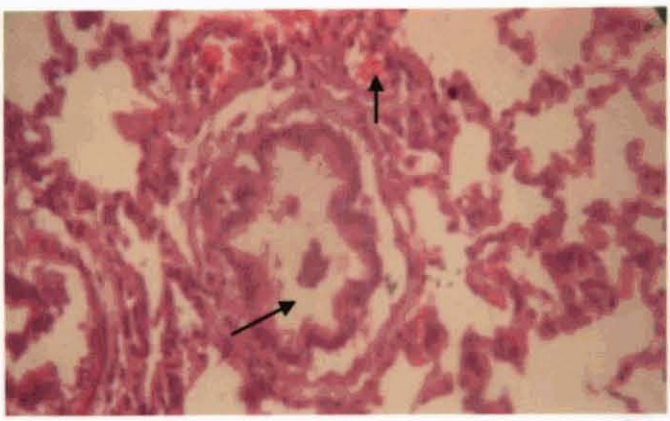

Fig. 18

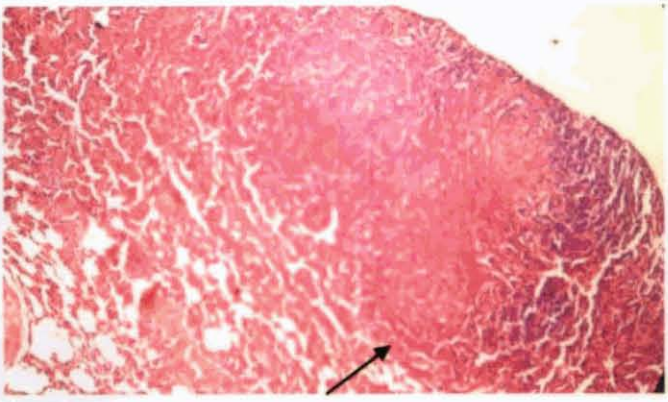

Fig. 19

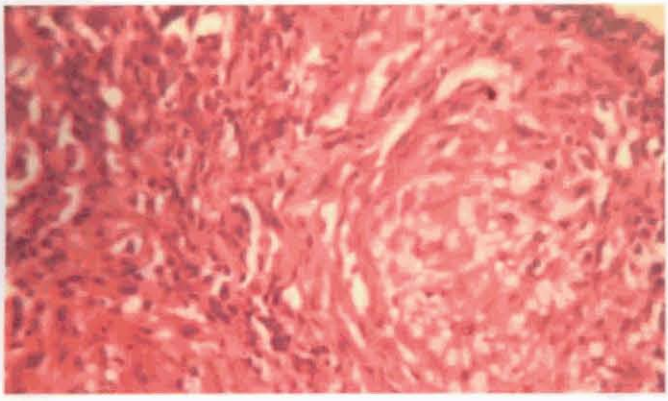

Fig. 20

Fig. 18 Lungs – Group II treated for two weeks
Desquamation of bronchial epithelium and congestion of interalveolar septa – H&E x 100

Fig. 19 Lungs - Group II treated for two weeks
Focal necrotic area surrounded by inflammatory reaction - H&E x100

Fig. 20 Lungs - Group II treated for two weeks
Proliferated fibroblast type of cells surrounded by infiltrated mononuclear cells – H&E x 400

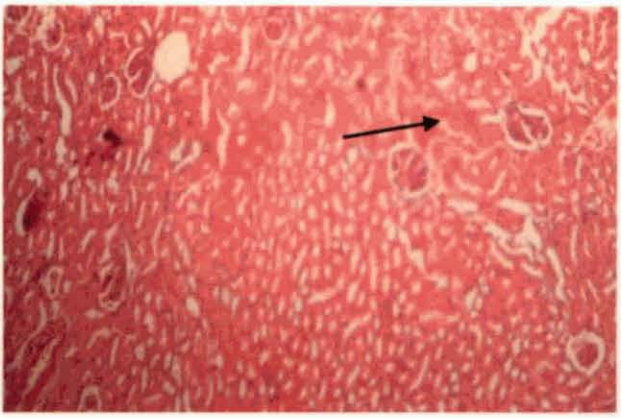

Fig. 21

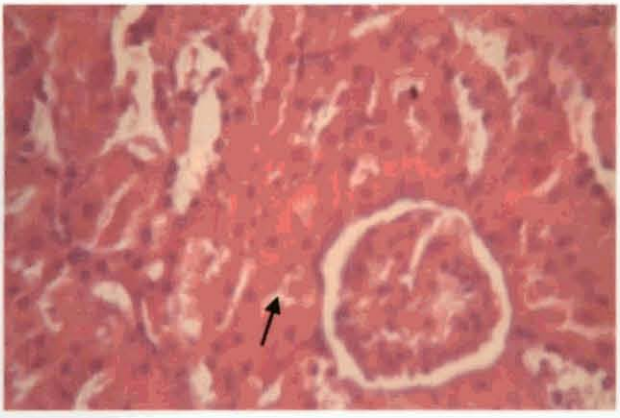

Fig. 22

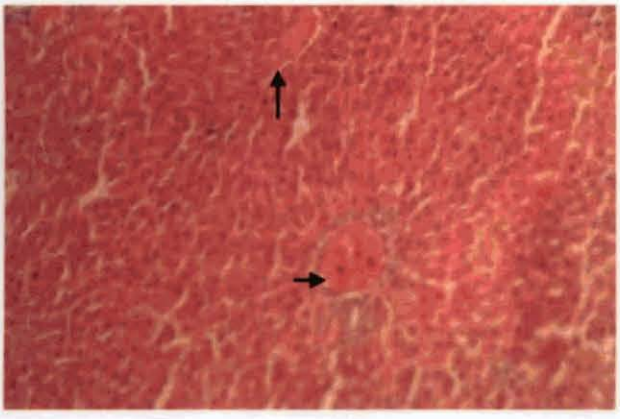

Fig. 23

Fig. 21 Kidney – Group II treated for two weeks

Focal areas of haemorrhage - H&E x100

Fig. 22 Kidney – Group II treated for two weeks

Narrowing of tubular lumen due to swollen tubular epithelium - H&E x 400

Fig. 23 Liver – Group II treated for two weeks

Congestion of central and portal veins - H&E x100

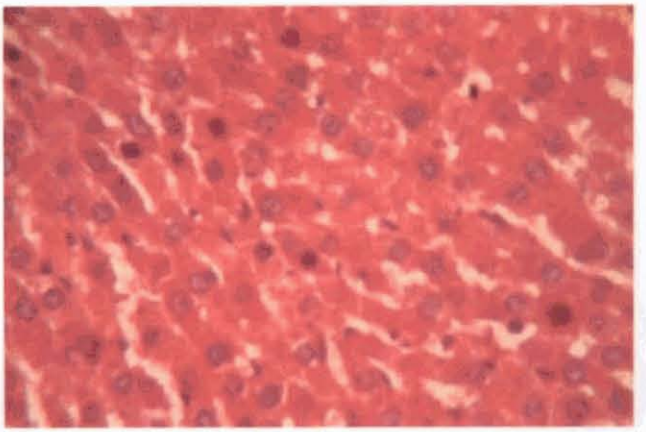

Fig. 24

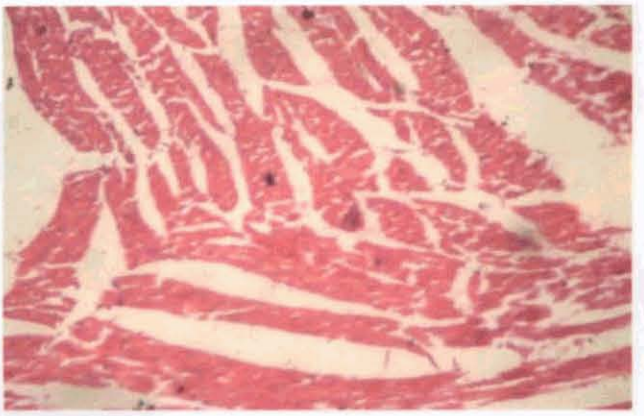

Fig. 25

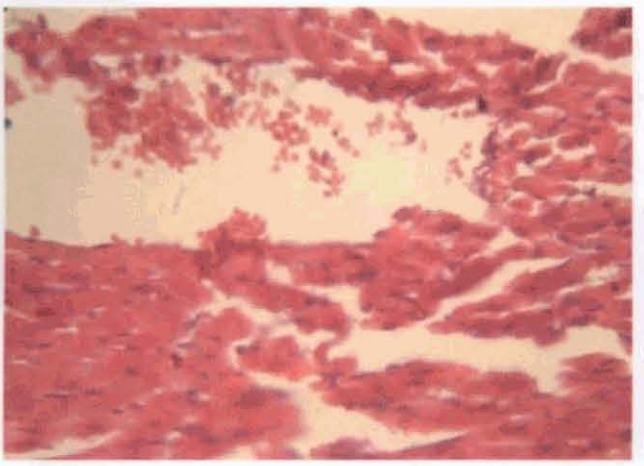

Fig. 26

Fig. 24 Liver – Group II treated for two weeks

Hepatocytes with granular cytoplasm - H&E x 400

Fig. 25 Heart – Group II treated for two weeks

Separation of degenerated cardiac muscle fibres – H&E x 100

Fig. 26 Heart – Group II treated for two weeks

Focal areas of haemorrhage – H&E x 400

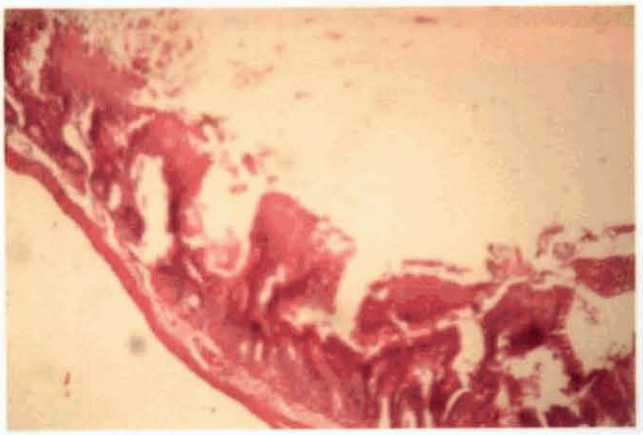

Fig. 27

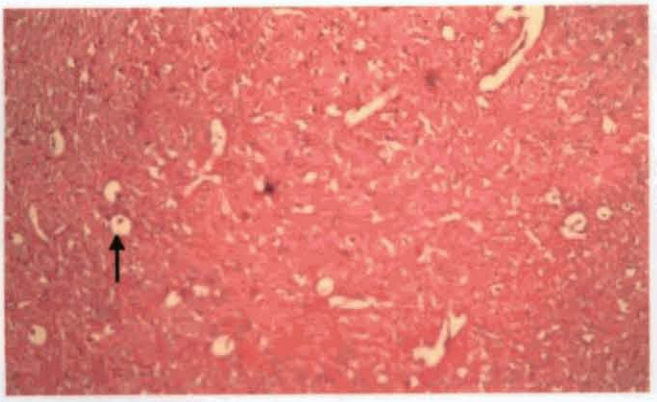

Fig. 28

Fig. 27 Intestine- Group II treated for two weeks
Necrotic changes at the tips of the villi - H&E x 100

Fig. 28 Brain – Group II treated for two weeks
Mild oedema - H&E x 100

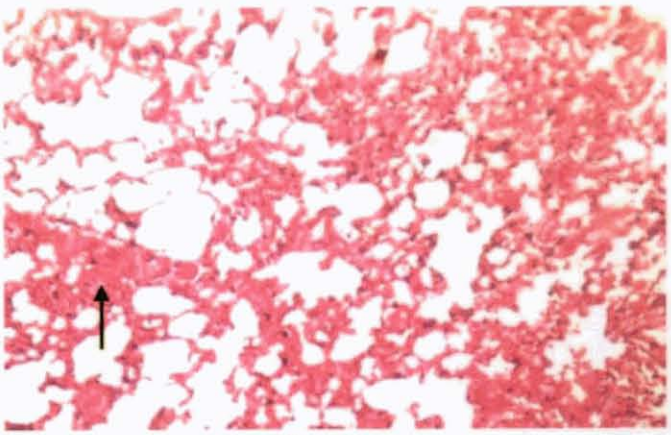

Fig. 29

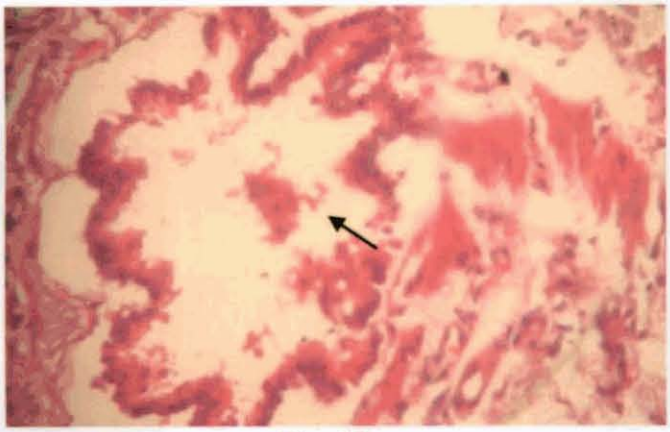

Fig. 30

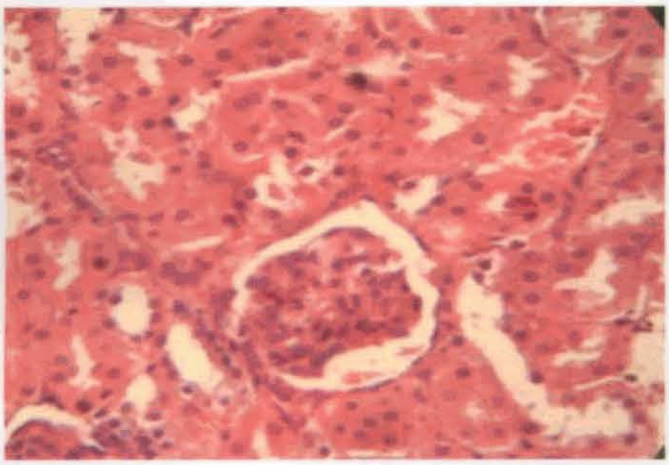

Fig. 31

Fig. 29 Lungs – Group II treated for four weeks
Thickened interalveolar septa - H&E x 100

Fig. 30 Lungs- Group II treated for four weeks
Desquamation of bronchial epithelium - H&E x 400

Fig 31 Kidney – Group II treated for four weeks
Severe degeneration and necrosis of tubular epithelium -H&E x 400

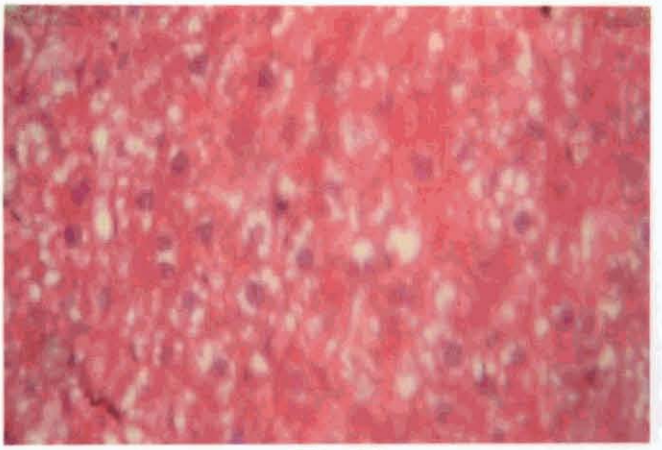

Fig. 32

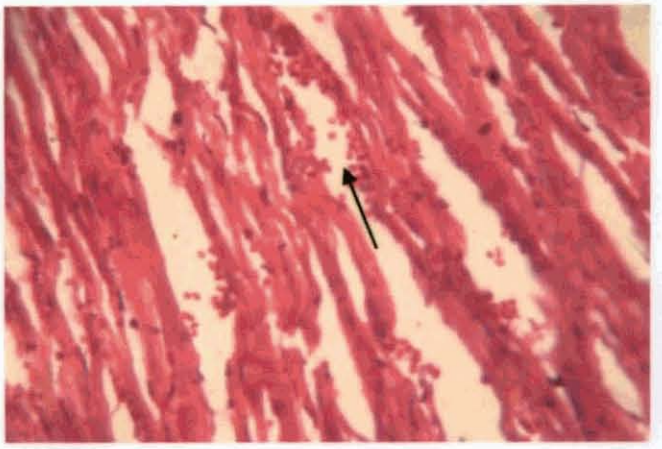

Fig. 33

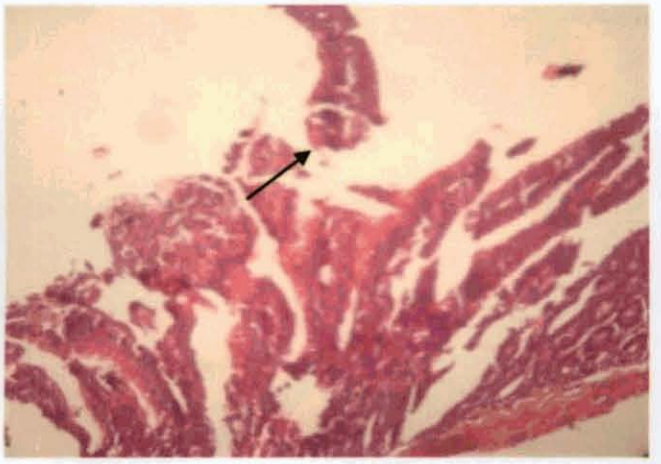

Fig. 34

Fig. 32 Liver – Group II treated for four weeks
Focal necrosis and vacoular degeneration - H&E x 400

Fig. 33 Heart - Group II treated for four weeks
Separation and fragmentation of heart muscle with haemorrhage - H&E x 400

Fig. 34 Intestine - Group II treated for four weeks
Necrosis at the tips of the villi - H&E x 100

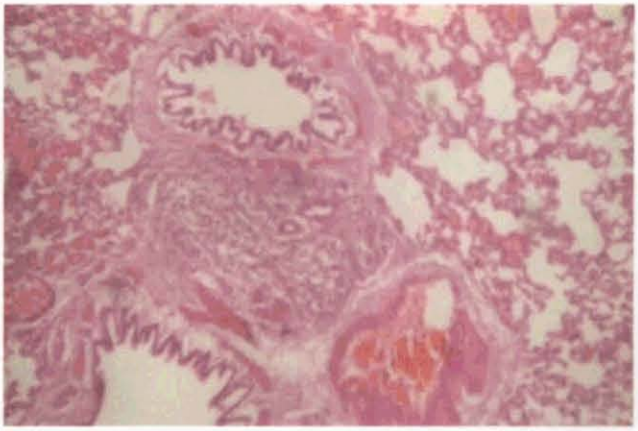

Fig. 35

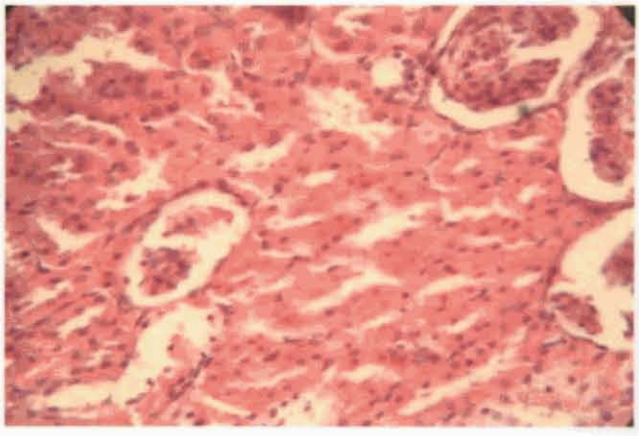

Fig. 36

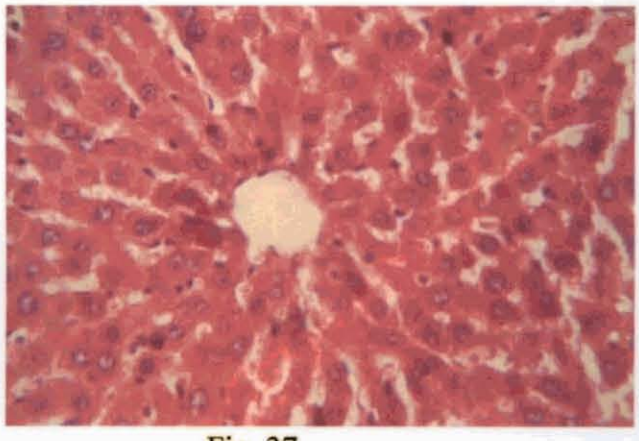

Fig. 37

Fig. 35 Lungs – Group III treated for two weeks
Intact bronchial epithelium and lymphoid aggregation, congestive changes present – H&E x 100

Fig. 36 Kidney - Group III treated for two weeks
Swollen tubular epithelium and focal necrotic changes – H&E x 400

Fig. 37 Liver - Group III treated for two weeks
Mild degree of degenerative changes devoid of any congestive changes H&E x 400

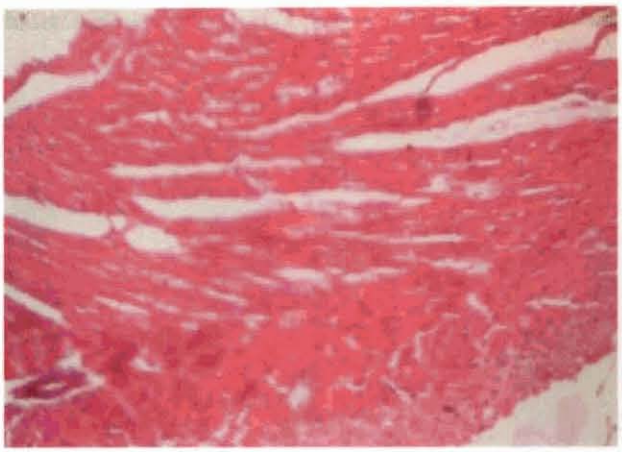

Fig. 38

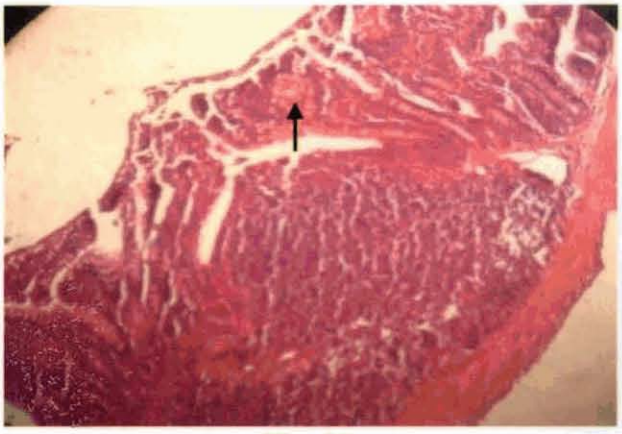

Fig. 39

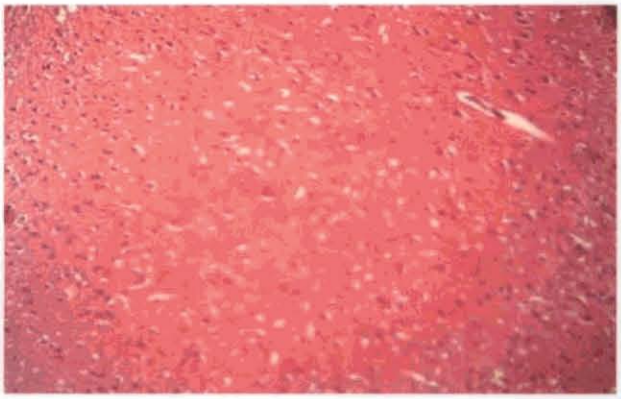

Fig. 40

Fig. 38 Heart - Group III treated for two weeks
Densely packed muscle fibres - H&E x 100

Fig. 39 Intestine- Group III treated for two weeks
Mild goblet cell hyperplasia with intact lymphoid aggregates- H&E x 100

Fig. 40 Brain - Group III treated for two weeks
No appreciable changes – H&E x 100

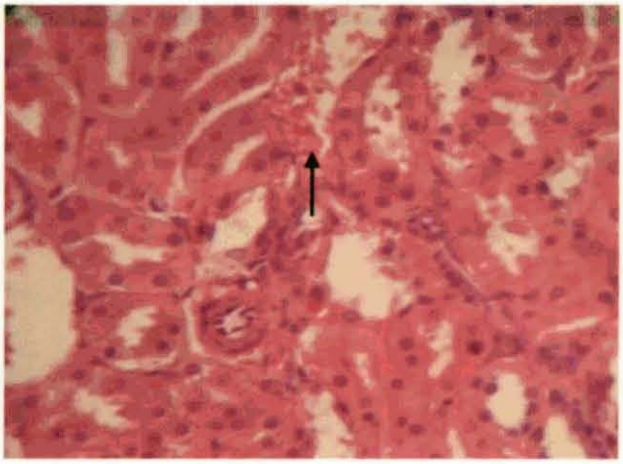

Fig. 41

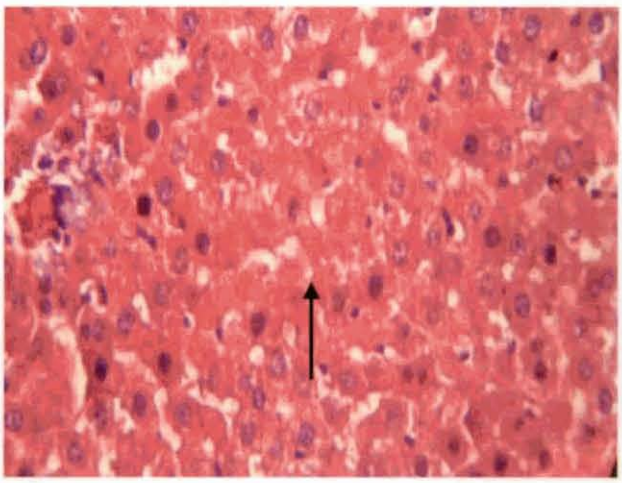

Fig. 42

Fig. 41 Kidney - Group III treated for four weeks
Mild degenerative changes and haemorrhage - H&E x 400

Fig. 42 Liver - Group III treated for four weeks
Focal necrotic changes – H&E x 400

Discussion

5. DISCUSSION

The present study was undertaken to assess the pathology of subacute paraquat toxicity and its amelioration by vitamin E in rats. Paraquat is a widely used non-selective contact herbicide in agriculture. It is a highly toxic compound for humans and animals which cause oxidative injury to vital organs as evidenced by the induction of lipid peroxidation, oxidation of the cellular NADPH, depletion of reduced glutathione and alteration in the antioxidant status resulting in ultimate degeneration of the tissues. The major cause of death in paraquat poisoning is respiratory failure due to an oxidative insult to the alveolar epithelium with subsequent obliterating fibrosis.

Vitamin E is well known for its antioxidant properties in biological membranes by physiochemical interaction between its phytol side chain and the fatty acid chain of polyunsaturated phospholipids. Considering the involvement of oxidative stress implicated in the toxicity of paraquat dichloride, the present study was designed to investigate the protective role of Vitamin E on paraquat toxicity in rats.

Adult female Sprague Dawley rats were used as experimental animals which were randomly divided into three groups of 12 each. Group I served as control group maintained on normal feed and water. Group II received paraquat dichloride at a dose rate of 25 mg/kg body weight orally three times per week for four weeks using a gastric tube. Group III received paraquat dichloride at a dose rate of 25 mg/kg body weight along with vitamin E at the rate of 200 mg/kg body weight orally three times per week for four weeks.

Animals were weighed and blood was collected on day zero and at weekly intervals for the estimation of serum parameters and haematological parameters. Six animals each from Group I, II, and III were sacrificed at the end of second and forth weeks. Weighed quantity of lungs and kidneys were collected

for estimation of lipid peroxides and reduced glutathione. Detailed gross and histopathological examination of all organs was done.

5.1 PHYSIOLOGICAL PARAMETERS

5.1.1 **Body Weight**

The experimental animals did not show any significant variation in body weight as compared with the control animals. Although, all the groups showed a gradual increase in body weight, Group II and III showed a reduced weight gain when compared with the control group. This reduced weight gain may be due to oxidative stress induced by paraquat dichloride. This is in agreement with the report of Hunter and Prahlad (1981) that the body weight was slightly decreased in one-day old rats treated with 25 mg paraquat per kg body weight, but the lung weight was not significantly lower than those of the control.

5.2 BIOCHEMICAL PARAMETERS

5.2.1 **Aspartate aminotransferase (AST)**

The activity of AST is a useful indicator of liver, heart, muscle and kidney function as it is located in the cytoplasm and released into the circulation after cellular damage.

A significant increase in the AST levels was observed in group II and III on day 14, 21 and 28 when compared to the control group. This is in agreement with Tamuli *et al.* (2004) as they observed an increase in AST levels in calves fed with paraquat sprayed grass. The SGOT, BUN and total serum protein levels increased significantly in experimentally induced paraquat toxicity in calf and goat (Tamuli *et al.*, 2009).

In the present study, the liver, heart and kidney showed histopathological lesions. Varying degrees of degenerative changes were noticed in these organs of

both group II and III. The intensity of these lesions was slightly less in Vitamin E treated group. The increase in serum concentrations of AST levels could be related to these lesions. The increase in AST levels was less in group III when compared to group II, but it was not a statistically significant difference. This is in accordance with the observations of Nahla *et al.* (2009) that the damage in the liver and kidney tissues were evident as elevated plasma ALT, AST, ALP, urea and creatinine in diazinon toxicity was partially counteracted by vitamin E administration

Higher activities of aspartate aminotransferase and gamma-glutamyltransferase induced by carbon tetrachloride were reduced by intramuscular injection of vitamin E in steers (Yonezawa *et al.,* 2005).

5.2.2 **Alanine aminotransferase (ALT)**

ALT is found mainly in the liver, but smaller amounts are also present in the kidneys, heart, muscles, and pancreas. A significant increase in the ALT levels was observed in treatment groups on day 7, 14, 21 and 28 when compared to the control group. Mostly, increases in ALT levels are caused by liver damage.

In the present study, histopathological lesions varying from mild degeneration to necrosis could be observed in the liver of group II animals. Group III animals also showed mild degenerative changes in the liver. Elevated levels of ALT could be attributed to this liver damage caused by lipid peroxidation.

Vitamin E caused a slight reduction in the elevated levels of ALT in group III. This is in agreement with the findings of Nahla *et al.* (2009) that the elevated plasma ALT in mice treated with diazinon was partially counteracted by vitamin E administration.

5.2.3 **Alkaline phosphatase (ALP)**

ALP is found mostly in the liver and in bone with some amount in the intestines and kidneys.

Rats in both group II and III showed a significant increase in ALP on day 7, 14, 21 and 28. This could be attributed to the damage to liver, kidney and intestine. Histopathological lesions varying from mild degeneration to necrosis could be observed in these organs. This observation in the present study is in contrast with the findings of Hoffman *et al.* (1987). They reported a decrease in the plasma alkaline phosphatase activity in American kestrel nestlings receiving 60 mg/kg of paraquat dichloride for 10 days.

Mild degenerative changes were noticed in vitamin E treated group also. A slight reduction in ALP levels was noticed in group III compared to group II. This is in accordance with report of Nahla *et al.* (2009) that the vitamin E administration partially reduced the elevated levels of ALP in mice intoxicated with diazinon

5.2.4 **Creatinine**

Creatinine is the break down product of muscle creatine phosphate. Creatinine is chiefly filtered out of the blood by the kidneys and a rise in blood creatinine levels indicated kidney damage.

A progressive and statistically significant increase in mean creatinine value was observed in group II and III on day 7, 14, 21 and 28 when compared to control group. This change is suggestive of a gradual development of renal lesions. This clearly indicated nephrotoxicity of the paraquat dichloride. This observations is in accordance with findings of Lamfon and Al-Rawi (2007) that the rats intoxicated with paraquat at a dose level of 1/36 LD50 (3.46 mg/kg BW) three times per week for three weeks caused marked elevation in serum creatinine

and blood urea nitrogen. But in toxicity study of paraquat sprayed grass in calves, Tamuli *et al.* (2004) observed a decrease in the creatinine values.

In the present study, a slight decrease in the level of creatinine was noticed in vitamin E treated group but it was not significant.

5.3 HAEMATOLOGICAL PARAMETERS

The parameters like Hb and PCV showed significant variations in treatment groups compared to control on day 7, 14, 21 and 28. The total leukocyte count showed a significant increase in treatment groups on day 28. The erythrocyte sedimentation rate and differential leukocyte count was found to be within the normal range.

The haemoglobin and PCV values were gradually decreased in both group II and III which might be due to haemolysis through lipid peroxidation. Vitamin E provided a slight protective effect against haemolysis in group III.

Decreased Hb and TLC were noticed in calves fed with paraquat sprayed grass. There was significant increase in neutrophil together with significant relative decrease in lymphocytes (Tamuli *et al.*, 2004). In experimentally induced acute paraquat toxicity in calves and goats, haemoglobin concentration, total leukocyte counts and neutrophils were increased, while lymphocyte count was decreased (Tamuli *et al.*, 2009).

Urban *et al.* (2005) noticed a genotoxic effect of paraquat even after exposure via dermal application. Paraquat at dose levels of 6, 15 and 30 mg/kg body weight was given to rats via the dermal route and number of micro-nucleated polychromatic erythrocytes was found to be increased in a dose dependent manner.

Niki *et al.* (1991) found out experimentally that vitamin E, a lipophilic chain-breaking antioxidant present within the membranes, suppresses the

oxidative damage of the membranes more efficiently. A statistically significant decrease in haemoglobin, red blood cells and hematocrit values in diazinon treated mice were partially remedied by vitamin E (Nahla *et al.*, 2009).

5.4 OXIDATIVE EFFECTS

Oxidative effect of the paraquat dichloride was determined by measuring the alterations in the values of lipid peroxides and reduced glutathione in tissue homogenates. In this study, it was observed that there was a statistically significant increase in lipid peroxides and a concurrent decrease in reduced glutathione in both lungs and kidneys.

5.4.1 **Lipid Peroxides**

Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids by reactive oxygen species. It is a molecular mechanism of cell injury leading to the generation of peroxides and lipid hydroperoxides which are aldehydes such as, malondialdehyde. Accumulation of these products in turn cause lipid peroxidation and cause serious damage to the cell membrane and leakage of intracellular enzymes resulting in loss of cell function and cell death. Estimation of thiobarbituric acid reactive substances is diagnostic indices of lipid peroxidation and tissue injury due to oxidative stress (Takenaka and Goto, 1994).

A significant increase in the lipid peroxide level of lungs and kidneys was noticed in both group II and III when compared to control group. A further increase in lipid peroxide level was noticed in animals sacrificed at the end of the experiment. This indicated that the paraquat dichloride is able to produce oxidative damage to the lungs and kidneys. Histopathological lesions observed in the present study confirmed lung and kidney damage. One of the comparable observations to this finding is that of Ray *et al.* (2007) who observed decreased glutathione level in blood cells, liver, lung and kidney and increased

malondialdehyde formation in a dose and time dependent manner in male albino rats after administering paraquat dichloride.

Anguelov and Chichovska (2004) also observed that paraquat increased the pulmonic, cardiac and hepatic concentration of hydrogen peroxide, conjugated dienes, lipid peroxides and malonyldialdehyde but it did not remarkably increase the concentration of these substances in the kidney which is in contrast to the results of the present study..

Sato *et al.* (2006) examined the lipid peroxide levels of homogenate prepared from the lungs, livers, kidneys and brains of 48 male mice treated with 30 mg/kg paraquat dichloride. A significant increase in the lipid peroxide level was identified only in the liver.

A mild decrease in lipid peroxide level noticed in group III may be due to scavenging of free radicals by vitamin E, as comparable to the observations of Susan *et al.* (2001) that the ameliorative effect of vitamin E on organo-phosphate induced oxidative stress by decreasing lipid peroxidation.

5.4.2 **Reduced Glutathione**

Glutathione provides protection against free radicals by scavenging them from the biological system. Reduced glutathione level reflects the antioxidant status of the tissues. Decreased glutathione levels have been considered to be an indicator of oxidative stress.

In the present study, group II and III showed statistically significant decrease in mean values of reduced glutathione when compared to control group. This indicated oxidative damage of the lungs and kidneys. A significant reduction in glutathione may be due to either decreased synthesis or increased utilization. This is in accordance with the finding of Bus and Gibson (1984) who identified that paraquat toxicity resulted in depletion of intracellular NADPH which was necessary for maintenance of glutathione in the reduced state.

Vitamin E supplementation could not provide a significant protection against lipid peroxidation in group III. This may be seen in the light of the report of Combs and Peterson (1983) that the acute toxicity of paraquat in chick was highly responsive to nutritional selenium status and not vitamin E status.

5.5 GROSS AND HISTOPATHOLOGICAL OBSERVATION OF THE TISSUES

None of the animals in the experimental groups revealed any clinical signs of toxicity except for one rat each from group II and III which became weak towards the end of experiment. Tamuli *et al.* (2004) studied the toxicity of paraquat sprayed grass in calves and the clinical signs noticed were lacrimation, frequent urination, frothy salivation, dehydration and emaciation along with muscular tremor and staggering gait.

Six animals each from Group I, II, and III were sacrificed at the end of second and forth weeks and detailed post mortem examination was conducted.

Gross lesions were almost similar in group II and III. The lungs showed varying degrees of emphysema and congestion. Moderate degree of congestion was noticed in kidneys and liver also. Gastric mucosa was slightly hyperemic in Group II animals.

The gross lesions in the present study were in accordance with the findings of Tamuli *et al.* (2003) who observed moderate to severe congestion, haemorrhages and varying degrees of emphysema in the lungs, slight enlargement of liver, dilatation of blood vessels in brain and mild to severe congestion of the small intestinal and abomasal mucosa in calves treated with paraquat.

Thurlbeck and Thurlbeck (1976) observed marked pulmonary congestion in female Sprague-Dawley rats treated with 25mg paraquat dichloride per kg body weight intravenously.

The lung, kidney and other organs showing lesions were subjected to detailed histopathological examination.

In rats treated with paraquat alone for two weeks, the lungs showed moderate degree of thickening of the interalveolar septa due to congestion and metaplastic changes of the alveolar epithelium to fibroblast cells. Bronchial lumen revealed desquamation of proliferated and necrotic epithelium. Proliferation of alveolar epithelium and mild depletion of the lymphoid tissue associated with bronchi were also observed. Oxidative damage of the lung was evident, as there was increase in lipid peroxides and decrease in reduced glutathione.

In few animals, focal areas of necrosis surrounded by proliferated epithelial cells, inflammatory cells and fibroblasts were also seen in lung parenchyma. These findings indicated the pulmonary toxicity of the compound and this type of lesion was observed in some of the rats fed with 500 ppm paraquat daily in their diet by Renate and Kimbrough (1971).

The paraquat was found to be nephrotoxic which was manifested by varying degrees of degeneration in renal tubules. An increase in the serum creatinine level and lipid peroxide level also reflected the kidney damage. Congestion and haemorrhage was present in the intertubular spaces. Liver showed cloudy swelling and central venous congestion. There was also an increase in the serum AST and ALT. These findings indicated the hepatotoxicity of the compound.

Mild degenerative changes in the stomach and necrosis of the villus epithelium of the intestine, goblet cell hyperplasia could be attribured to the localized response to the irritation. Intermuscular haemorrhage of heart and mild oedema in the brain were the other lesions observed.

In animals treated for four weeks, lesions became more severe, as evidenced by thickening of the interalveolar septa due to proliferation of the fibroblast cells and necrotic changes in lungs. A further increase in the lipid peroxide level of lung reflected this damage. There was reduction in the number of intact alveoli and some of the alveoli filled with edematous fluid containing erythrocytes and fibroblast-like cells. This finding is in accordance with the observations of Dearden *et al.* (1978). They observed similar lesions in the late stage of a case of human paraquat poisoning.

Kidney revealed severe degeneration and necrosis of tubular epithelium when compared with animals sacrificed at the end of second week. In the liver multifocal necrosis and sinusoidal congestion were observed. Mild degree of fatty change and haemorrhage between cardiac muscle fibres and severe oedema in the brain were also noticed. Stomach revealed degenerative changes of the glandular epithelium and in the intestine goblet cell hyperplasia and necrosis of the tip of the villi were noticed.

In the present study, lesions in the lungs, kidneys, liver, heart and brain are in accordance with the observations of Tamuli *et al.* (2003). The lesions in the stomach and intestine were almost similar to the observations of Dede *et al.* (2007). They observed mucosal ulceration and tubular hyalinization in stomach and loss of villi and glandular necrosis in intestine.

In vitamin E treated group, severity of lesion was found to be slightly reduced. This may be due to protective effect of vitamin E against lipid peroxidation. In rats treated for two weeks, the lungs revealed intact bronchial epithelium and active bronchi associated lymphoid nodules. Proliferation of fibroblasts was less. Focal tubular necrotic changes were noticed in the renal cortex. Liver showed slight degenerative changes, mild fatty change, focal necrotic changes, sinusoidal dilatation and Kupffer cell proliferation when compared with Group II. Slight degenerative changes were noticed in heart, stomach and intestine also. The slight reduction in the degree of desquamation,

proliferative changes along with the restoration of the active lymphoid nodules are indicative of a mild protective effect of vitamin E as described by the Jalili *et al.* (2007) who reported protective effect of vitamin E in endosulfan induced cardiotoxicity.

In rats treated for four weeks, a further reduction in intensity of lesion was noticed. A complete recovery was not possible. A slight reduction in the biochemical parameters like AST, ALT, ALP, creatinine, lipid peroxides and increase in the level of reduced glutathione supported this mild ameliorative effect of vitamin E. But this effect of vitamin E is not significant to manage pulmonary and renal disorders in subacute paraquat toxicity. These findings are in accordance with the observations of Combs and Peterson (1983). They observed that the acute toxicity of paraquat in chick was highly responsive to nutritional selenium status and not vitamin E status. Shekelle *et al.* (2004) studied the effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease and observed that vitamin E supplementation does not beneficially or adversely affect cardiovascular outcomes.

From the present investigation, it can be concluded that the vitamin E has only a slight ameliorative effect and hence not fully suitable for management of the renal and pulmonary disorders in subacute paraquat toxicity in rats.

Summary

6. SUMMARY

The present experiment was undertaken to study the pathology of paraquat toxicity in rats and to evaluate the ameliorative effect of vitamin E in paraquat toxicity.

Thirty six adult female Sprague Dawley rats weighing 150-200 g, divided in to three groups comprising twelve animals in each group, were used for the study. Group I served as control. Group II received paraquat dichloride at a dose rate of 25 mg/kg body weight orally three times per week for four weeks using a gastric tube. Group III received paraquat dichloride at a dose rate of 25 mg/kg body weight along with vitamin E at the rate of 200 mg/kg body weight orally three times per week for four weeks.

Blood was collected on day 0, 7, 14, 21 and 28 for estimation of ESR, Hb, PCV, TLC and DLC and serum was used for the estimation of AST, ALT, ALP and creatinine. Six animals each from Group I, II, and III were sacrificed at the end of second and forth weeks and detailed postmortem examination was conducted and lesions were recorded. Weighed quantity of lungs and kidneys was collected in chilled normal saline for estimation of lipid peroxides and reduced glutathione. Pieces of lung, kidney and other organs showing lesions were examined histopathologically.

Body weight of rats in all the groups showed a gradual increase throughout the experimental period. Though not significant statistically, the animals in group II and III showed a slight reduction in body weight gain when compared with the control group. Among the haematological parameters, Hb and PCV values were significantly decreased in treatment groups when compared to control group. The total leukocyte count showed a significant increase in treatment groups. The erythrocyte sedimentation rate and differential leukocyte count was found to be within the normal range. In vitamin E treated group, a slight increase in Hb and PCV values were noticed.

The AST, ALT, ALP and creatinine values showed significant increase in treatment groups which indicated damage to the liver, kidney and muscle. An increase in the levels of lipid peroxides and a concurrent decrease in reduced glutathione in lungs and kidneys were observed which indicated oxidative damage to these organs. When compared to paraquat alone treated group, a slight decrease in biochemical parameters was noticed in vitamin E treated group, but it was not statistically significant.

The animals in group II and III showed almost similar gross lesions. Moderate to marked degree of congestion and varying degrees of emphysema were noticed in the lungs. Moderate degree of congestion was noticed in kidneys and liver. Gastric mucosa was slightly hyperemic in Group II animals. On histopathological examination, in rats treated with paraquat alone for two weeks, the lungs showed moderate degree of thickening of the interalveolar septa due to proliferation of fibroblasts along with congestion, haemorrhage and desquamation of proliferated and necrotic bronchial epithelium. Focal areas of oedema were also noticed. Focal areas of necrosis surrounded by proliferated epithelial cells, inflammatory cells and fibroblasts were also seen in lung parenchyma in some of the cases of group II.

The kidneys revealed diffuse degeneration and necrosis of tubular epithelium along with congestion and haemorrhage. Liver showed degenerative changes varying from cloudy swelling to necrotic changes, central venous and sinusoidal congestion. Heart revealed intermuscular haemorrhage and mild fatty changes of muscle fibres. Stomach revealed mild degenerative changes and in intestine, goblet cell hyperplasia and necrosis of the tip of the villi were noticed. In the brain, mild oedema was noticed. In animals treated for four weeks, lesions became more severe.

In vitamin E treated group, severity of lesion was found to be slightly reduced. The lungs revealed intact bronchial epithelium and active bronchi associated lymphoid nodules. Proliferation of fibroblasts was less. Focal necrotic

changes were noticed in the renal tubular epithelium. Liver showed varying degrees of degenerative changes. Slight degenerative changes were also noticed in heart, brain, stomach and intestine. In rats treated for four weeks, a further reduction in intensity of lesion was noticed. But a significant difference was not noticed between group II and III.

From the present investigation, it is identified that ameliorative effect of vitamin E is not significant to manage pulmonary and renal disorders and it can be concluded that the vitamin E has only a slight ameliorative effect and hence not fully suitable for management of the renal and pulmonary disorders in subacute paraquat toxicity in rats.

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STUDIES ON THE AMELIORATIVE EFFECT OF VITAMIN E ON PARAQUAT TOXICITY IN RATS

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ABSTRACT

The present study entitled 'Studies on the ameliorative effect of vitamin E on paraquat toxicity in rats' was undertaken by administering the animals with paraquat dichloride and vitamin E for a period of 28 days. The weekly body weights, clinical signs, haematology and biochemical parameters were analysed. Of the twelve animals in each group, six animals were sacrificed at the end of second week and remaining at the end of forth week. The gross pathology and histopathology of various organs were studied. The oxidative damage of the lungs and kidneys was assessed by the estimation of lipid peroxides and reduced glutathione.

None of the animals in the experimental groups revealed any clinical signs of toxicity except for one rat each from paraquat alone treated group and paraquat plus vitamin E treated group which became weak towards the end of experiment. Although, all the groups showed a gradual increase in body weight, those animals treated with paraquat alone and those with paraquat and vitamin E showed a reduced weight gain when compared with the control group.

The AST, ALT, ALP and creatinine values showed a significant increase in treatment groups compared to the control. In vitamin E treated group, a slight decrease was noticed in these biochemical parameters. Hb and PCV values showed a significant decrease and the total leukocyte count showed a significant increase in treatment groups when compared to control group. There was no variation in the erythrocyte sedimentation rate and differential leukocyte count. In vitamin E treated group, a slight increase in Hb and PCV values were noticed. There was an increase in the lipid peroxides and concurrent reduction in the glutathione in the lung and kidney homogenate which indicated oxidative damage.

Gross lesions were almost similar in both treatment groups. The congestive changes were noticed in lungs, kidneys and liver. The histopathological changes noticed in paraquat alone treated group were

congestion, haemorrhage, focal areas of necrosis with proliferation of fibroblasts in the lungs, thickening of the interalveolar septa. The bronchi revealed desquamation of proliferated epithelium. Degenerative changes in the renal tubules, focal necrotic changes in the liver and varying degrees of degenerative changes in the heart, stomach, intestine and brain were the other lesions noticed. These lesions became more intensified in animals treated for four weeks.

In vitamin E treated group, a slight reduction in the intensity of lesion was noticed. The bronchial epithelium was intact. Mild proliferation of fibroblasts and congestive changes were also noticed in the lungs. Focal necrotic changes were noticed in the kidneys and liver. Mild degenerative changes were noticed in the heart and intestine. The study revealed that the vitamin E is not completely suitable for management of the renal and pulmonary disorders in subacute paraquat toxicity in rats.