In the present study, toxicity of cadmium acetate in male albino rat is evaluated by determining medium lethal value. The calculated sublethal value after exposure to cadmium acetate 3.3 mg/kg body weight in male albino rats. Present findings indicate that the toxicity of cadmium acetate depends on concentration and duration of exposure. Similar findings regarding concentration and exposure time relationship with the lethality have been reported by Sharma (2001) in albino rats. Similar findings have been reported by Macquiddly et al. (1941) in rats and mice; Gray et al. (1954) in rats; Carson et al. (1962); Cooper et al. (1966) in lower animals; Wagner et al. (1965) in rats, and Mustafa et al. (1980) in rats after exposure to nitrogen dioxide gas on other metals. Present findings gain support with the findings of Hine et al. (1970) who observed that animal mortality may not result from 1hour exposure to cadmium acetate but an increase of either time or concentration will lead to mortality in mice and rats.

Present study indicates that toxicity of cadmium acetate is due to its strong oxidising property in cells which causes edema and mortality due to penetrating power of cadmium acetate into the tissue. Present findings are in agreement with the findings of Chauhan, et al. (2002); Kaya et al. (1980); Mustafa et al. (1980); Pryor and Lightsey (1981); Pryor et al. (1982); Sagai and Ichinose (1987); and
Melamed et al. (2003) who reported the formation of free radicals and tissue damage by nitrogen dioxide gas and heavy metals.

In the present study, the activity responses are observed during the exposure of sub-lethal dose of cadmium acetate. Cadmium acetate effect in host body scratching, distress and restlessness which are due to toxic effect of cadmium acetate.

IMMUNOPATHOLOGICAL STUDIES

LIVER

Immunopathological studies in liver of Albino rat after sublethal dose of cadmium acetate treatment

Immunopathological changes observed in liver and spleen of control and cadmium acetate treated rats.

Immunopathological studies of cadmium acetate treated albino rat were studied after 35 and 70 days of post treatment.

During present investigation changes in the hepatocyte, sinusoid, central vein were observed after the cadmium acetate treatment in albino rat. In experimental group changes were observed cloudy swelling, focal collection of lymphocyte, oedema, pyknosis, karyolisis, karyorrahexis and karyokinesis. With cadmium acetate several works have been reported in the liver during cadmium acetate exposure.
As a result of pollutant intoxication various immunopathological changes have been reported. Schwartz and Otto (1925) have reported that continued ingestion of cadmium carbonate resulting in increase of leucocytes which in turn give rise to inflammatory reaction. Chauhan et al. (2005) Immunopathological alteration caused by A. galli infection and cadmium acetate toxicity in intestine of white leg horn chicks.

Changes in liver have been attributed due to disturbance of lipid metabolism and protein metabolism (Prodan 1933, Huges et al. 1944 and Karmaker 1989). The liver is the most important organ subjected to greater environmental stresses and cadmium intoxication. Presently effect of animal on Cd- induced liver damage has been reported. The result from this study demonstrate that the affect of Cd on albino rat more in 70 days of post treatment i.e. after 70 days showed more resistance to Cd-induced hepatotoxicity than 35 days treated rat. Similar findings were reported by the following workers (Goering and Klaasen, 1984; Yamano et al., 1998) observed damaging effects of Cd on younger rats. The central vein was found to be dilated. The dilated central vein might be regarded as a physiological response to cadmium toxicity of the tissue for more blood. It is a well established fact that regardless of the site of administration, hepatic concentration of cadmium via blood serum is very rapid (Lucis et al. 1969; Flick et al. 1971; Stowe et al. 1972 and Morselt et al. 1973).
The route of administration, hepatic concentration of cadmium via bloodstream is very rapid and one of the highest in magnitude as compared with other organs (Lucis et al. 1969; Flick et al. 1971; Stowe et al. 1972 and Morselt et al. 1973). The hepatocytes showed marked degree of degenerative changes. Several researchers have reported that hepatic cell injury was always involved after cadmium intoxication (Friberg et al. 1971; and Stowe et al. 1972). In the present study, the appearance of lesions in the liver due to cadmium, appeared to be dose and time dependent. The necrosis in the liver lobules was more pronounced and also in the vicinity of central vein were observed. The focal collection of inflammatory cells were observed very prominently after cadmium intoxication around the central vein and the portal region. The increase in the number of inflammatory cells showed immunological response to the toxic action of cadmium since these cells were phagocytic in action. The necrosis and the inflammatory reaction suggested the toxic action of cadmium. Hoffman et al. (1976) reported that the single parenchymal cell necrosis in liver was the end result of an integrated pattern of functional and structural damage produced directly or indirectly by cadmium acetate. They also described the focal collection in lymphocytes, necrosis of hepatocytes in the rats and suggested that probably this lesion also arose due to cadmium toxicity. The mechanism of Cd-induced hepatotoxicity has been investigated extensively. The initial damage is produced by the interaction with the vascular endothelium and degenerative changes in the
endothelium which lead to damage to surrounding hepatocytes (Nolan and Shaikh, 1986). The initial damage may be caused by a disturbance in homeostasis (Li et al. 1994). Liver injury occurs from inflammatory process that are initiated by the activation by Kupffer cells (Sauer et al. 1997; Dong et al. 1998). Activated kupffer cells release chemoattractants and activations of neutrophils and promote extensive tissue damage.

During the 35 days of cadmium acetate treatment degenerated hepatocyte were seen in throughout the section. We have reported that the important nutritional changes were seen and body weight were slightly decreased compared with control rats. This result was supported by the observation (Masumi et al. 1999 and Chauhan et al. 2007) of thioacetamide treatment on rats and cadmium acetate on white leg horn chicks.

The fatty change in the liver is caused by malnutrition and can be correlated to metabolism. In fatty changes (Prodan, 1932; Hughes et al. 1944; Kendall et al. 1945; Karmaker et al. 1986) of the liver cell was considered to be firstly damaged resulting in metabolism, impaired utilization of lipids followed by the appearance of fat in the cell. The change in hepatocytes causes injurious influence. It may now be suggested that the fat metabolism of hepatocytes might have been interfered by cadmium acetate administration which resulted in fat accumulation in hepatocytes.
The appearance of foci of necrosis in the liver was in agreement with the findings of Hoffmann et al. (1976). During the chronic study thick bands of fibrosis were fairly observed around the central vein as well as portal tract after 35 and 70 days of post treatment. This observation was similar to the acute and chronic experiment observed by Stowe et al. (1972).

**SPLNEEN**

The present investigation has revealed that cadmium treatment marked immunopathological changes in spleen of albino rat. The capsular wall of spleen was found to be quite thick and ruptured at various places whereas red and white pulp revealed inflammatory oedema with eosinophils and macrophages. The cadmium treated rats revealed marked immuno-pathological changes shows hyperplasia in follicles cells were observed. During present investigation due to formation of secondary lymphoid nodule. The appearance of secondary lymphoid nodule were spleen associated with an infective process as they were always found in the spleen treated rats but were absent in control rats (Thorobecke et al. 1957). Spleenic congestion and inflammatory and non-inflammatory oedema were observed in infected and treated group of albino rat, may be due to presence of scattered erythrocyte and lymphocyte. Depletion of lymphoid cells were observed in spleen of Proilar chicks (Stoev et al. 2000; Holovzka and Jurajda 1992; Bagurt et al. 1979; Tanigiuhit et al. 1977). Wedderburn (1974) observed hypertrophy of spleen. Macrophages living in the spleen sinuses would unfair
normal blood flow that may effect in rat. Similar changes were observed in spleen having parasitic infections (Rogers et al. 1975; Alikhan and Sibu, 1980).

Hyperplastic follicles contained germinal centers and lingible bodies. The peritoneal area of spleen were well developed and showed no depletion of lymphocyte (Chadr and Merovitch 1985).

Thus present studies have revealed immunopathological changes not only in intestine where the toxic substances absorption occurs but also in other visceral organs like liver, kidney and spleen. The effect of cadmium toxicity induces severe hypersensitivity reactions causing various immunopathological changes.

HAEMATOLOGICAL STUDIES

In present study, total RBC count, haemoglobin concentration and haematocrit value decrease significantly after exposure to sublethal dose of cadmium acetate. Decrease in total RBC count accompanied with haemoglobin concentration and haematocrit value is due to exposure of cadmium acetate which causes inadequate oxygenation of blood that acts as a stimulus for erythrocyte production and result in hypoxic polycythaemia in albino rats. Present findings support with the findings of Wilcox et al. (1993) who have observed erythropoiesis inducing polycythemia in humans after nitric oxide exposure. Kawata et al. (1998) and Walker et al. (2000) has also noted nitric oxide inhalation
causes chronic hypoxia stimulating erythropoiesis and resulting polycythemia in rats. Baskurt et al. (1980) and Mederois et al. (1983) also reported erythropoieting stimulation in students after exposure to air pollutants. Kalra (2001); Agarwal and Kalra (2003) have also reported erythropoietic stimulation causes polycythemia in albino rats after nitrogen dioxide exposure. Similar views regarding decrease in total RBC count, haemoglobin concentration and haematocrit value due to hypoxic polycythemia have been given by Furiosi et al. (1973) after nitrogen dioxide exposure in rats.

Similar to the present findings a decrease in total RBC count, haemoglobin concentration and haematocrit value have been reported by Friedman et al. (1973) in smokers; Nagai et al. (1982) in humans due to enzymatic reduction; Tirlapur et al. (1983) in smokers; Ernst et al. (1986) in volunteers due to environmental changes; Maejima et al. (1992) in rats after exposure to fuel and Pachauri (1992) in squirrel after exposure to nitric oxide gas.

Our data indicate the haematotoxic effects of cadmium acetate treatment after orally chronic exposure are dose and time dependent. The mechanism of cadmium induced anemia are not understood. The proposed mechanism include (A). Iron deficiency due to inhibition of iron absorption from the gastrointestinal tract.
In the present study, total WBC count increases significantly after 35 and 70 days exposure to cadmium acetate. The increase in total WBC count is due to accumulation of cadmium acetate which causes damage to tissue and capillary membrane accompanied with inflammation evidenced by migration of white blood cells to the site of tissue injury leads to the increase of total WBC count in peripheral blood resulting formation of immune globin in albino rats. Similar to present observation, a increase in total WBC count have been reported by Saxena (1998); Agarwal (2003) in rats after combined exposure to sulphur dioxide and nitric oxide and Kalra (2001) in albino rats after exposure to nitrogen dioxide gas. Similar observation regarding increase in total WBC count in rats have been given by Srivastava et al. (1984) after exposure to flyash; Alarie et al. (1972) after exposure to sulphur dioxide gas.

In the present study, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) increases significantly after 35 and 70 days exposure to cadmium acetate. A rise in these values of red cell indices is directly correlated with increase in total RBC count, haemoglobin concentration and haematocrit value causing hypoxic polycythemia in albino rats. This is in agreement with the findings of Thomas and Penney (1977); Penney and Bishop (1978) after carbon monoxide exposure and Kalra (2001) and Agarwal and Kalra (2003) who reported that MCV and MCHC were depressed during polycythaemia after nitrogen dioxide exposure in rats.
Furiosi et al. (1973) also noted polycythemia with rised MCV in rats and monkeys. Similar observations have been made by Baskurt et al. (1990) in military students after exposure to air pollution. Baskurt and Balkanci (1988) and Srivastava (1995) have reported increase in MCH and MCHC in rats after exposure to sulphur dioxide inhalation. Walter (1999) also observed increase in red cell indices after exposure to carbon dioxide in rats.

Blood vessels are common pipe-line through which enzyme blood components (R.B.C., W.B.C., platlets and eosinophil cells etc) and also an entire immune components (antigens, antibodies, B-cells, T-cells, macrophages, cytokines, IFNγ-1, TNF-1, TNF-γ etc.) are transported to cells are tissues. Interesting co-relation in the present study have been observed for the first time. The affinity of blood and immune component to become more relevant as both have common origin from HSC (Haematopoeitic stem cells) primary lymphoid organ the bone marrow.

In the present experiment the albino rat with treatment of cadmium acetate revealed leucocytosis with a significant rise in total leucocyte count. The rise in total leucocyte counts, lymphocytes, eosinophils, monocytes and basophils was obviously due to the immune responses against cadmium toxication in albino rats. The increase in eosinophil cells, leucocytes, monocytes, basophils shows strong inflammatory reaction in cell mediated immunity.
A fall of neutrophils were found in present study. Neutrophils are the most important cells involved in the Body's defence. Neutrophils are migratory phagocytic cells. Reduce number of neutrophils indicates the toxic effect on bone marrow (Ramnisood, 2003). Destruction of neutrophils in the peripheral blood due to toxic effect of cadmium could be the reason of reduce number of neutrophils (Ramnisood, 2003). Gill T.S. (1985) also reported a mild neutropenia in the cadmium exposed fish.

In the present study differential leucocyte counts revealed a significant rise in lymphocyte, eosinophil, monocytes and besophils. Lymphocytes are motile non-phagocytic cells. There are many subpopulation of lymphocyte which interact with each other and with cells of the monocyte macrophage series in maintainance of humoral and cell mediated immunity. Increase in lymphocytes indicated increase humoral immune response. Lymphocytes are cytotoxic cells when the level of toxic substances increase in the body then to neutralize the effect of toxic no. of lymphocyte increased. Gill T.S. (1985) also reported elevated small lymphocyte and besophil population and a mild neutropenia in the cadmium exposed fish.

The characteristic eosinophilic response observed in the present study seems to have appeared as a result of liberation of powerful toxin. Eosinophils also possesses phagocytic capacity (Ramnisood, 2003). Increase in eosinophil also indicated increased cellular immune response. Schuwerack P.M. et al. (2003) also reported a significant increase in the eosinophil in thymus in infected and
cadmium exposed infected carp fry. Rise in eosinophils in cells reveals inflammatory reaction factor.

Monocytes are also motive phagocytic cells. These are involve in the phagocytosis and catabolism of necrotic material. In the present study number of monocytes increased. Monocytes together with neutrophils form a system of phagocyte through out the body which act as a first line of defence against infection/toxicant.

Increase number of monocyte showing its antitoxic activity. Monocyte play a role in the immune system by processing certain antigens. Gail M. et al. (2002) also reported increased number of monocytes in three metal treatment of rainbow troat. In the present study basophil percentage increased. Gill T.S. et al. (1985) also reported elevated basophil population in cadmium exposed first. Increase in besophils also shows cellular immune response. Besophils are chemotoxic blood cells in case of cadmium toxicity their number increased.

**BIOCHEMICAL STUDIES**

The toxic effects of metal arise from their action on biological system. The potential site is blood where various biochemical parameters are affected. In the present study a significant fall was revealed in the serum protein level. Serum protein level in rat treated with sublethal dose of cadmium acetate revealed a constant decline in comparison to be untreated control rat throughout the experimental hypoproteinemia was observed in albino rat.
The glucose is formed from the digestion of carbohydrates. The values of glucose into blood takes places in two ways (i) conversion of liver glycogen to blood glucose (glycogenolysis) (ii) Formation of blood glucose by liver from non carbohydrate sources viz. amino acids, pyruvate, lactate (Glucoseoneogenesis). Glucose-6-phosphate dehydrogenase is a key enzyme in glycolysis and catalyze Glucose-6-phosphate to 6 phospholactone through HMP pathway and provides NADPH to stimulate glucose metabolism.

In the present study, blood glucose increases and significantly after 35 and 70 days exposure to sublethal dose of cadmium acetate. An increase in blood glucose level is due to accumulation of toxic metal cadmium acetate that causes tissue injury seems to stimulate glycogenolysis and gluconeogenesis resulting in hyperglycaemia in albino rats. It is a well known fact that any type of tissue injury and stress condition stimulates sympathetic branch of nervous system to release epinephrine. In the liver, epinephrine stimulates glycogenolysis and gluconeogenesis in muscles from lactate and release glucose from liver and muscle into the blood, and this stimulation was determined by increase activity of G-6PD. (Rhoades and Pflanzer, 1992) present findings again support with the findings of Tierney et al. (1974) and Mustafa et al. (1978 and 1980) who reported that increase in glucose level is stimulated via HMP pathway by increased G-6PD value in albino rats after exposure to nitrogen dioxide gas. Penney (1993) and Walter (1999) have also observed increase in blood glucose level due to release of
epinephrine resulting in increased glycogenolysis. Similar findings have been noted by Young and Knelson (1973); Ospital (1976); Tierney et al. (1977); Mustafa et al. (1979, 1980a, 1980b) and Basett (1986) who observed increase in blood glucose level due to lung injury which stimulated increase production of lactate resulting in increase in gluconeogenesis in albino rats. Similar views regarding an increase in blood glucose level have been given by Srivastava et al. (1984) and Hoffman et al. (1987) who reported damage of lung tissues resulting in hyperglycemia due to increase in glycogenolysis in albino rats.

Further, an increase in blood glucose value is correlated with increase in serum total lipid in albino rats due to increase in lipolysis. In adipose tissue, epinephrine stimulates lipolysis, i.e. breakdown of triglycerides to fatty acids and glycerol, Glycerol is taken up by liver and converted to glucose. (Chatterje, and Shinde, 2000). Present findings gain support with the findings of Hayes et al. (1976) and Sharma (1997) who reported the an increase in blood glucose level with increase in serum total lipid due to lipolysis in albino rats.

In the present study serum total cholesterol increases significantly after exposure to sublethal dose of cadmium acetate. An increase in serum total cholesterol level is correlated with increase in blood glucose accompanied with increase in glucose-6-phosphate dehydroegnase leads to increase in lipolysis in albino rats. The oxidation of glucose by HMP pathway due to increase in G-6PD provides NADPH to increase the synthesis of fatty acids which inturn enhance
cholesterol level in albino rats. Present findings are in accordance with the findings of and Mustafa et al. (1980) in rats after exposure to nitrogen dioxide gas.

An increase in serum total cholesterol level may also be associated with reduced lipoprotein lipase activity which is presumably the basis of hypercholesteemia as lipase activity represent a group of enzymes which are associated with the hydrolysis of lipid contents. Sharma (2001) have also reported the increase in serum total lipid due to decrease in lipoprotein lipase activity after nitrogen dioxide inhalation in albino rats.

Another possible explanation for the increase in total cholesterol level is due to exposure of cadmium acetate which causes tissue damage and lipid peroxidation. Lipid peroxidation is a basic deteriorative process in living system involving polysaturated fatty acids and phospholipids in cellular membrane which in turn elevate serum total cholesterol in albino rats. Similar findings to present findings, increase in serum total cholesterol due to lipid peroxidation causing pulmonary injury have reported by Shimizu et al. (1986); Sagai and Ichinose (1987); Gelzleichter (1992); Elsayed (1994) and Sharma (2001) in rats after exposure to nitrogen dioxide gas.

Similar findings to the present findings, Thomas et al. (1968); Hazzard et al. (1969); Tapple (1970); Mudd and Freeman (1977); Blank (1978) and Mustafa et al.
(1980) have reported an increase in serum total lipid in rats after exposure to nitrogen dioxide gas.

In the present study, serum total protein decreases significantly after exposure of sublethal dose of cadmium acetate at both the intervals. The decrease in total serum protein is due to digestive inflammation in albino rats which accompanies epithelial cell injury by cadmium acetate exposure resulting in an increase in epithelial and capillary membrane permeability which causes leakage of proteins from serum to site of tissue injury, leads to decrease in serum total protein value in albino rats. Present findings are in accordance with the findings of Selgrade et al. (1981); Denicola et al. (1981); Mustafa et al. (1984); Menzel et al. (1984); Guth and Mavis (1985); Shimizu et al. (1986) and Mohsenin (1991) who stated that decrease in serum total protein is indicative of inflammation due to extensive pulmonary injury in rats after exposure to nitrogen dioxide gas. Sherwin et al. (1968); Blair et al. (1969); Gardner et al. (1969); Sherwin and Richters (1971); Sherwin and Carlson (1973) and Drozdz et al. (1976) also reported decrease in serum total proteins in guinea pigs after exposure to nitrogen dioxide gas.

Serum acid phosphate is found in bones, teeth, blood and cells. It plays an important role in acid base regulation by kidney, energy transfer and is a constituent of phospholipids, nucleic acids, lipoproteins and nucleotides. It is
determined from serum as ester phosphates in red cells are hydrolysed and form inorganic phosphate (Varley, 1980).

In the present study, serum acid phosphate increases significantly after 35 days and 70 days of sublethal exposure to dose highly significantly after 35 days exposure. The increase in serum acid phosphate is correlated with hypoxic polycythemia due to accumulation of cadmium acetate which causes increase in red cell count and haemoglobin concentration as phosphate uptake by liver and muscle cells may be at the expense of erythrocyte leading to decreased red cell which causes an increase in the oxygen affinity of haemoglobin and can result in low serum phosphate level in albino rats. In support of present findings, increase in serum acid phosphate due to hypoxia have been reported by Macmillan (1978) in rats after carbon monoxide exposure; Hoppe et al. (1978) in rats after carbon monoxide exposure; Hoppe et al. (1982); Yoshino et al. (1986) and Minura and Knox (1994); Meyer et al. (1994) and Mimura (1995) in rats.

Further increase in serum acid phosphate is correlated with increase in blood glucose level which results in formation of phosphorylated hexose intermediates and cause acute shift of phosphates into cell and induce hyperphosphataemia (Varley, 1980).

A very slightly significant increase in serum alkaline phosphate after 35 days and 70 days of exposure to cadmium acetate is due to increase in
concentration and time period of toxic substance which induces hyperphosphatemia in albino rats.

Enzymes are proteins present in the globulin protein of serum. They are not confined solely to the serum but are present in various portions of cells, though the amount in different organs can vary widely. When an organ is damaged, a greater amount of enzyme leak out into plasma. The extent of the rise in serum activity of these enzymes depends on the concentration of enzymes in the tissue and on the severity of damage. The rate at which an enzyme leaks from damaged tissue is affected by location of the enzyme in the cells and by change in the permeability of the cell membrane. In the present study, the activity of certain serum enzymes viz. Glucose-6-phosphate Dehydrogenase (G-6PD), lactate dehydrogenase (LDH), serum lipase, serum alkaline phosphatase, serum acid phosphatase, serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) increases significantly after exposure to the sublethal concentration of cadmium acetate. This is an indication of tissue damage in albino rats due to exposure of toxic metal.

The phosphatases are enzymes which catalyse the splitting of phosphoric acid from monophosphoric esters. Alkaline phosphatases are localized in hepatocytes, lining of bile canaliculi and sinusoidal membranes. The enzymes are bound to intracellular microsomal membranes. In the present study, alkaline phosphatase increases significantly after 35 and 70 days exposure to cadmium
acetate. A significant increase in the activity of serum alkaline phosphatase is attributed to the damaging effect of cadmium acetate on biliary lining, the isoenzymes of hepatic origin arising from the lining of bile canaliculi and also from sinusoidal surface of hepatocytes, much of which escapes into circulation causing increase in enzyme activity (Duncan and Prasse, 1986). Present findings are in accordance with the findings of Gregory (1985) and Shimizu et al. (1986) who stated that serum alkaline phosphatase activity increased due to injury of liver after exposure to nitrogen dioxide gas. Mustafa and Tierney (1978) and Bhattacharya and Gautam (1992) have also reported increase in serum alkaline phosphatase activity due to hepatotoxic action of metal causing damage to liver cells in rats after exposure to cadmium acetate. Similar views regarding increase in serum alkaline phosphatase activity have been given by Srivastava et al. (1984) after flyash exposure; Kaplan (1986) and Seetharam et al. (1986) after exposure to sulphur dioxide and Walter (1999) after exposure to carbon dioxide in rats.