CHAPTER 2

Effect of Deltamethrin 1% + Triazophos 35% EC on Haematological and Biochemical Profile in Wistar Rat

Many of the standard safety evaluation studies are on repeated dose studies such as sub chronic, chronic, reproduction toxicity designed to characterize the effect of the test chemicals upon multidose exposure to experimental animals. Sub chronic studies evaluate health hazards that may result from repeated exposure which is on a limit part of the test animals' lifetime and also evaluate dose related effect on survival, body weight, feed consumption hematology, serum chemistry and gross and microscopic organ changes and assess the potential **(Piccirillo, 1999).**

The results of clinical pathology test are used to identify general metabolic and pathological process. Although specific "diagnosis" or toxicological mechanisms are frequently identified, the test results help to direct further study by narrowing the possibilities. Alterations in test result are rarely the only evidence of biologically important adverse toxicological effect. Biochemical tests are necessary to generate information concerning carbohydrate, lipid and protein metabolism, renal function, liver function, hepatocyte injury and electrolyte balance **(Hall, 1992).** The observation on the haematological changes in laboratory animals exposed to environmental chemicals or new pharmaceutical agents is important in the overall assessment of the risks and hazards of potential human or animal exposure. Blood is a complex mixture of cells that respond in different ways of various toxicological insults. For example, chemically induced anemia, may results from direct myelotoxicity, hypoproliferative bone marrow, oxidative stress, non-oxidative destruction, immune hemolysis, ineffective erythropoisis, or blood loss. The effect of chemicals are not only restricted to the blood cells destruction or the inhibition of the haemopoiesis. Some agents stimulate hemopoiesis and other affect the function of blood cells, resulting in depressed or an enhanced function **(Luind, 2000).**

Variations in the constituents of blood cells are useful in the diagnosis of ailments affecting the organ such as liver and kidney. **Janbaz and Gilani (1995)** reported that elevation of transaminases and alteration in fat metabolism might induce hepatotoxicty. In another investigation by **Gupta *et al.*, (2001)** revealed significant elevation in ALT, AST and ALP

activities in a Clinico-hemo-biochemical studies on paracetamol at 500-mg/kg b.wt is indication of hepatotoxicity. Seven days treatment of phosphamidon, monocrotophos and endosulfan revealed changes in biochemical parameters (increase in MCV and MCHC and decrease in WBC, RBC, Hb and PCV) on fish **(Dhembare and Pondhe, 2000).** Haematological and biochemical study carried out with endosulfan (5, 10 and 15 mg/kg/b.wt.) on male rats (15 /30 days) resulted decrease in RBC, PCV, and Hb, MCH, and MCHC, and significant increase in WBC. Serum Biochemical parameters such as ALT AST, ALP and bilirubin increased and showed hepatotoxic effect of endosulfan **(Choudhary and Joshi, 2002).**

Various experiments were carried out to study hematological and biochemical profile of synthetic pyrethroids as well as organophosphate insecticides in various models of animals and reported adverse effect. Significant change in serum transaminase was reported in animals treated with deltamethrin and other pyrethroids **(Shukla, 1991; Ayub shah and Gupta, 2001; Garg *et al.*, 1997).** Exposure of synthetic pyrethroids (fenvelerate) caused significant decrease in serum albumin and glucose level and increase of serum transaminases activity in cockerels **(Singh *et al.*, 2001).**

Effect of haemo-biochemical profile with the exposure of pendimethalin suggested low toxicity of compound **(Ayub Shah *et al.*, 1994).** Another experiment showed toxic effect of pendimethlin (24 and 48 mg/kg/day) following chronic exposure in rat which was investigated by **Ayub Shah *et al.*, (1998).** Treatment did not affect blood glucose, BUN, total protein, ALT, AST and relative weight of kidney. Significant reduction (<0.05) in total erythrocyte count was found with the slight reduction in Hb, TLC, ALC at value at 48 mg/kg body weight.

Repeated oral administration of synthetic pyrethroid insecticide cypermethrin (0.25 mg/kg/day) for 21 consecutive days in male buffalo calves produced significant reduction in serum triglyceride, HDL cholesterol, VLDL cholesterol and increase in total bilirubin, urea and uric acid **(Sandhu *et al.*, 2001).** A comparative repeated dose oral exposure of two different synthetic pyrethroids, cypermethrin (0.25mg/kg/day) and deltamethrin (0.2mg/kg/day) in buffalo calves for 21 consecutive days induced significant inhibition of ChE activity (44.4% and 30.7%) with significant increase in AST (24.9 and 25 5%), ALT (19.8 and 28.7%), ALP (39 and 28.2%)and ACP (48.5 and 31.2%). Results suggested a higher toxicity of cypermethrin as compared to deltamethrin **(Kaur and Sandhu, 2001).** Another experiment conducted by **Kumari *et al.*, (2002)** in buffalo calves with the single exposure cypermethrin at 40 and 80 mg/kg body weight produced a significant inhibition of cholinesterase enzyme at 40 mg/kg body weight. Significant increase in AST, ALT, GLU and

BUN was recorded at 40 and 80 mg/kg body weight. **Misra *et al.*, (1985)** reported significant inhibition of cholinesterase on worker exposed to organophosphate.

A repeated dose (90 days exposure) study on lambda-cyhalothrin 2.5%EC in Wistar rats caused elevation of inorganic phosphorus, albumin and significant ($p>0.01$) decrease of chloride ions at 3000 ppm on day 45. At the end of exposure period (on day 90), increase of inorganic phosphorus, calcium and creatinine levels at 500, 3000 and 20000 ppm was observed with the reduction of chloride ions. Exposure did not alter the AST activity and BUN, Total protein, total bilirubin, sodium and potassium concentrations **(Kirshnappa *et al.*, 2000)**

Estimation of haematological profile exhibited significant reduction in haemoglobin, erythrocyte count, PCV, Platelets count and increase in MCV and MCH in a subacute toxicity study of Nuvan (Dichlorvos 76% EC) which is an organophosphate insecticide in rats **(Sahai, 2001). Kaur *et al.*, (2000)** studied blood biochemical and pathomorphological alteration in goat due to subacute (28 day) exposure chlorpyriphos at the dose levels of 0.5 and 1.0 mg/kg b.wt. Dose and time dependent inhibition was observed in both the erythrocyte's and serum ChE activity. Values of serum total protein, cholesterol, creatinine and transminase activity were significantly raised. Histopathological evaluation evoked effect in kidney and liver suggest severe toxicity of chlorpyriphos.

Effect of organophosphate insecticide on blood chemistry and serum enzymatic profile in lactating rats was investigated with monocrotophos (0.3, 0.6 and 1.2 mg/kg b.wt) exposure for 2 weeks pre-mating through out gestation and lactation period. Significant elevation of serum cholesterol, urea nitrogen, GOT, GPT and alkaline phosphatase were found with significant decline in blood glucose and cholinesterase activity which showed its toxicity to pups even at low dose **(Adilaxamamma and Reddy, 1995).**

Single exposure of cholrpyriphos at the rate of 20, 50 and 100 mg/kg b.wt revealed 52, 62 and 72 % serum cholinesterase inhibition, respectively. Following two consecutive days exposure the percent inhibition was 73, 77 and 80 and following three days exposure inhibition was 72, 81 and 85% at above doses **(Verma *et al.*, 2002).** Another experiment with single oral exposure of dichlorvos (DDVP) at the dose levels of 33.75, 67.5 and 101.25 mg/kg body weight in male mice showed significant inhibition in plasma and RBC's cholinesterase activity Inhibition of plasma cholinesterase was more marked and dose dependent. However, the recovery of plasma and RBC's cholinesterase were virtually complete 48 hours post treatment **(Bhatnagar *et al.*, 1994).**

Shiva Kumar *et al.*, (2002) reported no significant or dose dependent alteration on haematological parameter in rats administered deltamethrin orally daily once at the dose levels of 3, 6 and 12 mg/kg body weight for consecutive 28 days. Clinical signs of hyperxcitability and salivation were noted in the high dose group. **Shiva Kumar *et al.*, (2000)** suggested that the emulsifiable concentration are more toxic than the pure compound in an acute study conducted with deltamethrin in male and female rats. In another experiment of synthetic pyrethroid in albino rats treated with the fenpropathrin 10% EC at the dose levels of 5.916, 2.958 and 1.479 mg/kg body weight for 90 days did not reveal any significance variation in total erythrocyte count. Significant reduction was observed in Hb, PCV, MCV, MCH and total leukocyte count. However an increase was observed in neutrophil value **(Bhelonde and Ghosh, 2004).**

Histopathological examination of mice treated with chlorpyriphos 40% EC ($1/10^{th}$ of LD_{50}) for 10 days revealed congestion and degenerative changes of liver and hyperemia in kidney **(Revathi and Sunitha, 2000).** In other experiment, synthetic pyrethroid fenvalerate 35 % EC induced various cellular and sub cellular changes in the liver and kidney of squirrel, *Funambulus pennanti,* when treated with single dose at sub lethal concentration (0.004%) in the gram and found to be highly toxic to mammalian species **(Tripathi *et al.*, 2001).** Evaluation of ultrastructural changes in liver in rats treated with paralidoxima following acute organophosphate (methamidophos) poisioning resulted serious changes in hepatocytes and organelles. However the changes were reversible with the treatment of paralidoxima **(Satar *et al.*, 2004).** A chronic toxicity (8 weeks) study with synthetic pyrethroid (fenvalerat @ 20ppm), organophosphate (monocrotophos ethamidophos @ 2ppm) and chlorinated hydrocarbon (endosulfan @ 2ppm) in broiler chicks revealed increase in alkaline phosphatase and reduction in serum total protein with the effect on pathophysiology **(Garg *et al.*, 2004). Bhelonde and Ghosh, (2004)** evaluated pathological effect of fenpropathrin 10% EC (100, 120, 140, 160, 180 and 200 mg/kg body weight) in rats following single exposure. Grossly, congestion and haemorrhages were observed in most of the organ that succumbed to the acute toxicity. Microscopic examination revealed lesions in liver (fatty changes and centrilobular necrosis of hepatocytes), lung (focal oedema, congestion and emphysema), Kidney (tubular generation and necrosis) and brain (perineuronal oedema and congestion)

The above studies indicated the effect of synthetic pyrethroid and organophosphate on erythrocyte composition, serum biochemistry, as well as on enzyme activity. Test material is a combinational product of synthetic pyrethroid (deltamethrin) and organophosphate insecticide (triazophos) and both deltamethrin and triazophos are toxic whereby directly or indirectly affecting the ecosystem including mankind. In the present scenario of growing use

of formulated and combinational agro-products, which fore tells their popularity for giving multiple outputs to a cost effective input. Their easy availability and ready utility are their market driving forces. It has been observed that a compound in the presence of the other may exhibit a property, which is not obtained from there parent compound. It is necessary to predict the toxic effect of a combination insecticide i e. synergistic, additive or antagonistic. However, it is also reported that organophosphate accentuates the effect of deltamethrin **(Haines *et al.*, 2001; Environmental Health criteria 97, 1990).** Thus the following experiment was planned to evaluate the effect of deltamethrin1%+tiazophos 35% EC on haematological, serum biochemical and histopathological alteration.

In present investigation, hematological parameters such as white blood corpuscles (WBC), red blood corpuscles (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet Count (PLT) and clotting time were considered for the study. The biochemical parameters studied were serum glucose (GLU), alaline aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) alkaline phosphatase (ALP), cholinesterase (ChE), total protein.(T.PRO), albumin (ALB), Cholesterol (CHO) and electrolytes (Ca^{2+}, Na^{+}, K^{+} and Cl^{-}).

MATERIAL AND METHODS

Four groups of animals comprising 5 males and 5 females were administered with repeated dose deltamethrin 1% + triazophos 35% EC through oral gavage at the dose levels of 0 (G1: control), 10 (G2: Low dose), 20 (G3: Mid dose) and 30 (G4: high dose) mg/kg body weight/day. Male animals were dosed for 15 weeks (70 days prior mating, during mating and thereafter till sacrifice). A female animal were dosed for 14 days (2 weeks) prior to mating and then dosing was continued till mating, throughout gestation and lactation period. All the animals were starved for overnight prior to dosing and 3 - 4 hours' post dosing except females (feeding was *adlibitum* during gestation and lactation period). Deltamethrin 1% + triazophos 35% EC was dissolved in distilled water and administered at the dose volume of 10-ml/kg-body weight. Control group animals were maintained in similar condition with the treatment of distilled water

Prior to sacrifice, blood was collected by orbital sinus puncture **(Riley, 1960).** Changes in haematological parameters were determined using haematological analyzer (Sysmex K 1000). The procedure specified in the Sysmex Operation's Manual (1988) was followed. Serum Biochemical Parameters such as GLU, ALT, AST, BUN, T. PRO, ALB, Ca^{2+}, CHO,

ALP and ChE were analyzed using fully automatic analyzer, Hitachi 902 (Hitachi System Limited, Japan) Na^+ and K^+ analyzed using flame photometer (Chemito 1020) and Cl^- was analyzed on Erba Chem 5 plus semiautomatic analyzer. At the end of the experiment all the animals were euthanised by carbon dioxide asphyxiation and subjected to gross pathological examination. Histopathological examinations were carried out for the target organs such as liver, kidney, spleen, thymus and lymph node **(Godkar, 1994).**

Statistical Analysis

Group mean and standard error were calculated, data were analyzed using Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison for significance between the control and treated groups.

RESULTS

Serum alanine aminotransferase (ALT) in male rats of all treated groups and in female rats at 20 and 30 mg/kg body weight was significantly higher ($p \leq 0.01$) than control **(Table 2.1 and 2.2; Figure 2.1).** Dose dependent increase in serum aspartate aminotransferase (AST) was observed in the both male and female rats. The changes observed in male rats were statistically significant ($p \leq 0.05$) at high dose group in male rats and mid ($p \leq 0.05$) and high ($p \leq 0.01$) dose group of female rats **(Table 2.1 and 2.2; Figure 2.1).**

Increase of serum alkaline phosphatase (ALP) with increase of dose levels was observed in treated groups when compared with the control. The increase in serum alkaline phosphatase was significant at the dose level of 20 and 30 mg/kg body weight of both the male and female rats **(Table 2.1 and 2.2; Figure 2.2).** Dose dependent and significant inhibition in serum cholinesterase (ChE) was found in male and female rats of all treated groups **(Table 2.1 and 2.2; Figure 2.2).**

Serum glucose concentration and level of serum blood urea and nitrogen (BUN) was significantly increased ($p \leq 0.01$) in male and female rats of all the treated groups when compared with the control **(Table 2.1 and 2.2; Figure 2.3).** Elevation of cholesterol was significant in male rats of all dose groups and in female rats of high dose group **(Table 2.1 and 2.2; Figure 2.4).** Mean values of serum Albumin (ALB) in male and female rats were increased with increase of dose levels over the control and was significant ($p \leq 0.05$) in male rats of high dose group **(Table 2.1 and 2.2; Figure 2.4).** Exposure of deltamethrin 1% +

triazophos 35% EC increased total protein with the increase of dose levels in male and female rats when compared with the control **(Table 2.1 and 2.2; Figure 2.4).**

Mean value of calcium in all treated groups (both sex) was found to be comparable with the control group. Dose dependent increase in sodium (Na^+) and potassium (K^+) level and decrease in chloride (Cl^-) level was found in both the male and female rats when compared with the control **(Table 2.1 and 2.2; Figure 2.5 and 2.6).**

Dose dependent reduction in erythrocyte count (RBC) was observed in both the male and female rats exposed to deltamethrin 1% + triazophos 35% EC as compared to the control. Decrease erythrocyte count in male rats of G3 and G4 was statistically significant ($p<0.01$), However, the changes observed in female rats was significant at high dose group **(Table 2.3 and 2.4; Figure 2.7).**

Consistent decrease in hemoglobin (Hb) concentration with increase in dose level was noted in male and female rats of treated group over the control and found to be statistically significant ($p<0.05$), in male rats of high dose group **(Table 2.3 and 2.4; Figure 2.8).** Significant reduction in the percentage of haematocrit (HCT) was induced by the deltamethrin1%+triazophos 35%EC in male rats of high dose group (30 mg/kg b.wt) and in female rats of all treated group when compared to control. Effect observed in male rats of low and mid dose group that was dose dependent **(Table 2.3 and 2.4; Figure 2.8).**

Dose dependent decline in mean corpuscular haemoglobin (MCH) was found in female rats treated with deltamethrin 1% + triazophos 35 % EC. However, the values observed were not statistically significant. The level of mean corpuscular haemoglobin (MCH) in male rat was similar to the control **(Table 2.3 and 2.4; Figure 2.9).** Mean corpuscular haemoglobin concentration (MCHC) in male and female rats was statistically significant at the dose level of 30 mg/kg body weight/day. Level of MCHC at the dose 10 and 20 mg/kg body weight was similar to the control **(Table 2.3 and 2.4; Figure 2.10).** Mean alteration was observed in values of mean corpuscular volume (MCV) when compared with control **(Table 2.3 and 2.4; Figure 2.9).**

No significant difference in mean value of white blood corpuscles (WBC) was found among male rats of the control and treated groups **(Table 2.3 and 2.4; Figure2.7).** However, female rats treated with deltamethrin 1% + triazophos showed a dose related (not significant) increase in WBC count. A dose dependent decrease in platelet count and increase in clotting time was observed in present study **(Table 2.3, and 2.4; Figure 2. 10).**

Gross and histopathological evaluation of the rats treated with delatmethrin1% + triazophos 35% EC were carried out at the termination of the experiment. Gross pathological evaluation revealed treatment related changes in liver (Mottling/hepatomegaly) and kidney (blotched/patchy congestion). Histopathological evaluation revealed lesions in liver such as eosinophilic foci, necrotic foci, hypertrophic foci and patchy haemorrhagic spot **(Figure 2.11 – 2.13).** However, kidney showed pathological manifestations like dilated pelvis, papillary atrophy/nephritis and hyperplasia of pelvis **(Figure 2.14- 2.18)**

DISCUSSION

The Nephritic syndrome, which is always the result of glomerular injury, is characterized by hypercholesteremia, and perhaps hyperadrenocorticism may also be associated with increased cholesterol concentration **(Hall, 1992; Godkar, 1994).** It was reported that the exposure of organophosphate increases serum cholesterol in rats **(Hanafy *et al.*, 1991).** Hypercholesteremia due to pesticides may play a role in the pathogenesis of arteriosclerosis **(Patelski, 1976).** Animals subjected to the test substance showed a significant hypercholesteremia which might be due to chemical induced stress. A resultant increase was found in both the cortical and medullary activity of the adrenal. **Kaur *et al.* (2000)** made a similar observation in a sub-acute study in goat following chlorpyrifos exposure. Findings are also in agreement with significant elevation in cholesterol reported, in lactating rats due to exposure of monocrotophos at 0.6 and 1.2 mg/kg body weight **(Adilaxamamma and Reddy, 1995).** Thus, it may be concluded that Hypercholesteremia induced in the current study might be due to triazophos intoxication.

Blood urea and nitrogen assay tests provide valuable information about the status of kidney function and also about the magnitude of the defect. Kidney function test generally gets affected by pre renal (decreased plasma value, dehydration, decreased blood flow, cardiac failure), renal (condition affecting glomular filtration rate, tubular function, or any changes in vascular system) and post renal condition such as reduction in effective filtration pressure of the glomeruli **(Godkar, 1994).** In the present experiment, the compound dose of deltamethrin and triazophos exhibited dose dependent increase in blood urea and nitrogen in treated rats. Hepatic dysfunction, parenchymatous damage to kidney and/or increased catabolism of protein are mainly responsible for elevation of blood urea nitrogen **(Finco, 1989).** Organophosphorous compound is known to cause nephrotoxicity in rats **Adilaxamamma and Reddy, (1995). Durdrikova *et al.*, (1992)** reported increase in the level of blood urea nitrogen in sheep exposed to cypermethrin. Moreover, one of the recent studies by **Kumari *et al.*, (2002)** on rat provide ample evidence of pyrethroids induced

hepatic dysfunction. The elevated levels of BUN in the treated rats therefore, could be a result of the test compound induced renal and/or hepatic dysfunction

Alkaline phosphatase (ALP) is excreted through biliary system. ALP is localized in liver and biliary obstruction leads to increase ALP levels in blood indicating liver damage. Elevated ALP values are also noticed whenever liver is affected by conditions like tuberculosis, and carcinoma **(Godkar, 1994).** Damage to organs like liver, small intestine, spleen, lungs and kidney may cause an elevation of alkaline phosphatase in blood **(Kaur and Sandhu, 2001).** In present study, significant increase was observed in serum alkaline phosphatase of rats treated with deltamethrin 1% + triazophos 35% EC at doses of 20 and 30 mg/kg b.wt/day which was dose dependent. Alteration detected by the present study might be attributed to the hepatic and renal alterations. **Adilaxamamma and Reddy (1995)** reported elevation of alkaline phosphatase due to effect of an organophosphate insecticide monocrotophos on blood chemistry and serum enzymatic profile of lactating rat and pups. Buffalo calves trated with cypermethrin and deltamethrin also registered an Increase in ALP **(Kaur and Sandhu 2001).**

Aspartate aminotranferase (AST) and alanine aminotranferase (ALT) assay tests are simple, reliable and important for the detection of hepatic changes. Both the transaminases are presents in liver cells. Alanine aminotranferase (ALT) is cytosolic enzyme and aspartate aminotranferase (AST) is predominantly mitochondrial enzyme. The rise in serum levels is related to the rate and extent of liver cell necrosis. Elevated levels of both AST and ALT activities are commonly found in liver disease. The increase in ALT was observed due to greater hepatocellular changes **(Godkar, 1994).** The elevation in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood has been used as indicator of hepatocellular damage and increased membrane permeability **(Evans and Heath, 1988; Cornelius, 1987).** Rats exposed with deltamethrin and triazophos in the present investigation revealed a significant increase in serum aspartate aminotransferase and alanine aminotransferase activity. Significant increase observed in serum AST and ALT activity in present study could be possibly due to chemical injury to hepatic parenchyma and/or to other vital organs. Similar observations were also made by **Mohhamad and Adam (1990)** in goat exposed to fenvalerate. Such findings had also been reported by **Kaur and Sandhu (2001)** who observed elevation of AST and ALT in buffalo calves with the treatment of cypermethrin and deltamethrin. **Kumari *et al.* (2002)** also reported significant increase in AST and ALT activity in single exposure of cypermethrin at 40 and 80 mg/kg body weight. Significant elevation in transminases activity was reported with the exposure of monocrotophos by **Adilaxamamma and Reddy, (1995).** A dose dependent, significant increase in serum AST and ALT activity was reported in a repeated dose toxicity study

following chlorpyrifos in goat **(Kaur *et al.*, 2000).** Significant elevated trends in serum transminases due to deltamethrin and other pyrethroids was also reported **(Shukla, 1991; Ayub Shah and Gupta, 2001; Garg *et al*, 1997).**

Serum glucose concentration depends upon intestinal absorption, hepatic production and tissue uptake of glucose. Variety of hormones influence the balance between hepatic production and tissue uptake. Insulin is the primary factor responsible for uptake of glucose by tissues. Hepatic gluconeogenesis is stimulated by glucagon and glucocorticoids. Further, glycogenolysis is stimulated by glucagons and catecholamines. And these actions tend to increase serum glucose level **(Hall, 1992).** It was also suggested that glucose level may increase in different conditions such as diabetes mellitus, hyperthyroidism, hyperpituitarism, adrenocortical hyperactivity and occasionally in certain hepatic disorders **(Godker, 1994).** Glucocorticoids inhibit the ability of insulin to mediate the recruitment of glucose transport protein from the cell interior to the cell surface. It also causes decrease in number of insulin receptors and the affinity of receptors for insulin **(Nodrenstrom *et al.*, 1983).** Significant increase in serum glucose concentration was observed following oral administration of deltamethrin 1% + triazophos 35% EC in the present investigation which might be attributed to the increase of secretion of catecholamines from adrenal medulla and/or increase in circulatory Glucocorticoids as reported by **Kaur and Sandhu (2001)** increase of blood glucose was observed in buffalo claves following cypermethrin (0.25 mg/kg body weight/day) and deltamethrin (0.2mg/kg body weight/day). **Ayub Shah and Gupta (2001)** also observed increased glucose concentration in rats exposed to permethrin at 120 mg/kg body weight. **Kumari *et al.*, (2002)** reported similar finding following single exposure of cypermethrin. However, **Adilaxamamma and Reddy, (1995),** reported a decline in blood glucose in rats due to organophosphate intoxication. Therefore, it is logical to surmise that the hyperglycemia observed in the treated rat might be due to the pyrethroids component of the test article used in the current study.

An important function of the serum protein is to maintain osmotic balance between the circulatory blood and tissue space. Albumin is of major importance for maintaining serum osmotic pressure. A significant rise in the serum protein value was observed in the present study. **Tizard (1996)** reported synthesis of acute phase protein in blood during the initial stage of tissue reaction due to release of interleukins, which might be responsible for hyperproteinemia. **Godkar (1994)** noticed increase in total protein during multiple myeloma, liver disease or in number of chronic exposures such as tuberculosis, rheumatoid arthritis, subacute bacterial endocarditis and lupus erythematous. Hyperproteinemia induced by deltamethrin and triazophos in present study, might be due to multiple myeloma as suggested in subacute study of chlorpyrifos **(Kaur *et al.*, 2000).** Currently observed

increase in albumin level was suggestive of toxic hepatitis or hepatic necrosis. These observations are in agreement with the findings of **Krishnappa *et al.*, (2000)** following the treatment of lambda cyhalotrin.

Electrolytes are the most important substances, which influence the distribution and retention of body water. Sodium and potassium are the predominant osmotically effective electrolytes. Several pathophysiological conditions are known to alter serum levels of sodium *viz.*, severe dehydration, diabetes insipidius, salt poisoning, Cushing's Syndrome etc. Toxic elevation of serum potassium (hyperkalemia) is observed in the case of renal failure, advance dehydration, shock and Addison's disease and causes cardiac and central nervous system depression, mental confusion and weakness of respiratory muscles **(Godkar, 1994).** Potassium plays an important role in impulse conduction and muscle contraction of heart where by hypokalemia/hyperkalemia both cause cardiac arrhythmia and sudden death as reported by **Joshi, (1999)**. In metabolic acidoses due to the exchange of extracellular hydrogen ions and intracellular potassium ion, increase in serum potassium concentration was observed by **Hall, (1992)**. Biochemical evaluation of treated rats exhibited significant and progressive increase in sodium and potassium values. Present investigation also revealed significant decrease in the level of chloride in serum which affects metabolism like water balance and acid balance. Hypochloremia observed in the treated rats might be associated with gastrointestinal HCl losses as reported by **Godkar (1994). Krisnappa *et al.*, (2000)** also reported decreased chloride concentration along with no change in Na^+ and K^+ in rats treated with lambda-cyhalothrin.

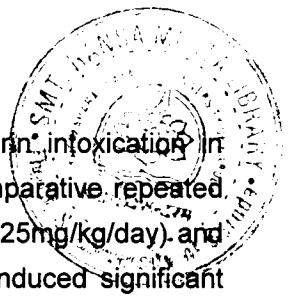
Cholinesterases (ChE) are widely distributed across animal species **(Ecobichon, 1996).** Cholinesterase (ChE) in nervous system regulates excitation by hydrolyzing the receptor bound neurotransmitter Acetyl choline (Ach). They are found at synapses, neuromuscular and myotendinous junction, cerebrospinal fluid, central nervous system, neuron cells bodies and axon, skeletal and smooth muscles **(Silver, 1974).** Cholinesterases are also present on the surface of erythrocytes, lymphocytes and platelets of mammals **(Husain, 1994).** Organophosphates exert their insecticide action by their ability to inhibit acetyl cholinesterase at cholinergic synapses resulting in the accumulation of acetylcholine which in turn leads to exaggerated neural transmission. A number of cholinesterase exists in the tissue of animals (i.e. nerves, blood cells, serum, liver etc.) and also in the tissues of insects, which are capable of hydrolyzing cholinesters. Amongst the many cholinesterases the acetylcholinesterase (AChE, acetylcholine hydrolase, EC 3.1.1.7) and butyryl cholinesterase (BChE, Acylcholine acylhydrolase EC 3.1 1 8) are often used in the toxicological evaluation of cholinesterase insecticides (**Wilson, 2001).** The source of erythrocytic AChE is bone

marrow while the serum enzyme called BChE comes from the liver. Erythrocytic AChE and serum BChE are known to be inhibited by organophosphates **(Verma *et al.*, 2002).**

Organophosphorus derivatives act by combining with and inactivating the enzyme acetylcholinesterase (AChE). The inactivation of cholinesterase by cholinesterase inhibitor pesticides allows the accumulation of large amounts of acetylcholine, with resultant widespread effects that may be separated into 4 categories: (1) Potentiation of postganglionic parasympathetic activity. (2) Persistent depolarization of skeletal muscle (3) Initial stimulation following depression of cells of central nervous system (4) Variable ganglionic stimulation or blockade **(Dreisbach, 1987)**. ChE are classed among the B-esterase enzyme inhibited by OPs and possess a serine catalytic site **(Ecobichon, 1996; Wilson, 2001).** Other B-esterase includes the broad class of carboxylesterases, one of which is neuropathy target esterase (NTE) that is associated with organophosphate-induced delayed neuropathy (OPIDN) However, all compounds are not capable to cause organophosphate-induced delayed neuropathy **(Ehrich and Jortner, 2001).** Organophosphate primarily affects the nervous system through inhibition of cholinesterase. **(El-Din Bayoumi, *et al.*, 2003).** Cholinesterase (ChE) in nervous system regulates excitation by destroying the neurotransmitter ACh **(Silver, 1974).**

In the present study a significant inhibition in the serum cholinesterase was observed. It has been reported that organophosphates inhibits cholinesterase activity in both single and repeated dose toxicity. Blood biochemical analysis revealed significant inhibitory effects on erythrocyte and serum cholinesterase activity (82 – 85%) at 24 h in goats treated with single dose chlorpyriphos at 200 mg/kg-body weight **(Kaur *et al.*, 1998).** Repeated dose toxicity of chlorpyriphos resulted 84.5% inhibition of serum ChE and 77.7 % inhibition of erythrocyte cholinesterase at 1.0 mg/kg body weight/day in goats **(Kaur *et al.*, 2000).** Another experiment of chlorpyriphos in rats also dropped serum cholinesterase activity 85% in 100-mg/kg b.wt. **(Verma *et al.*, 2002).** Inhibition of ChE activities in RBC and plasma (more marked) were also reported by **Bhatnagar *et al.* (1994)** due to exposure of dichlorvos in mice. Single oral administrations of 1.0, 2.5 and 5.0 mg/kg body weight of triazophos produced mild to moderate toxic symptoms of anticholinesterase poisoning in buffalo calves **(Sandhu and Bal, 1998)**. Triazophos inhibited dose dependent erythrocyte cholinesterase, (50 3-82.4%) and plasma carboxylesterase (37 4-54.5%).

Results of the present study show that deltamethrin 1% + triazophos 35% EC also inhibits serum ChE activity in rats. Inhibition in activity of ChE of rat's serum was significant ($p \leq 0.01$) and dose dependent. Results obtained in repeated dose for 150 days of triazophos exposure in buffalo calves **(Sandhu and Bal, 1998)** are supporting the current findings of



cholinesterase inhibition. Inhibition of cholinesterase due to cypermethrin intoxication in buffalo calves is also reported by **Vanketeshwarlu *et al.*, (1997).** A comparative repeated dose oral exposure of two different synthetic pyrethroids, cypermethrin (0.25mg/kg/day) and deltamethrin (0.2mg/kg/day) in buffalo calves for consecutive 21 days induced significant inhibition of ChE activity **(Kaur and Sandhu, 2001)**. Another investigation by **Kumari *et al.* (2002)** also revealed significant inhibition of cholinesterase in buffalo calves with a single exposure cypermethrin at 40 mg/kg body weight.

Oral treatment of gravid female rats with methyl parathion from gestation days 6 to 20 resulted in significantly lower AChE activity in the blood and brain of dams. Pups from these dams showed suppression of blood and brain AChE activity (**Bevrouty *et al.*, 2001**). Nuvacrone, a fast acting organophosphorus pesticide, was tested on adult males and pregnant females of mouse revealed inhibition of ChE (**Abd el Aziz *et al.*, 2003**). Chlorpyrifos-methyl was given as a single oral dose to lactating females and their corresponding pups was able to produce a significant decrease in the activity of brain and serum AChE of both the dams and their pups. **(El-Din Bayoumi, et al., 2003).**

An anticholinesterase agent inhibits the activity of the cholinesterase enzyme and so is an important marker to assess the exposure of an individual to chemicals, especially those, which are neurotoxicants and cholinesterase inhibitors **(Taylor, 1996).** The most sensitive indicator of an effect of cholinesterase inhibition is the depression of cholinesterase activity in plasma and erythrocytes **(WHO, 1986).** In present study, blood cholinesterase activity was determined in serum that showed significant inhibition. Serum cholinesterase (ChE) is reported to be inhibited in many pathological conditions such as liver diseases, anemia, acute infections diseases, cardiac failure and renal diseases **(Long, 1975)** as well as by exposure with number of OP compounds. Results of the present study and reported literature **(Sandhu and Bal, 1997; Sandhu and Bal, 1998)** clearly indicate that serum BChE/and erythrocyte AChE are sensitive biomarker for organophosphates and other class of compounds.

The current study revealed that the combination product of deltamethrin 1% + triazophos 35% EC inhibit serum cholinesterase enzyme activity at different dose levels. Inhibition was dose dependent and had severe effect on cholinesterase. Inhibition of this enzyme is often used as an index to certain toxic effect of organophosphorus and carbamate insecticides. Significance of inactivation of cholinesterase by synthetic pyrethroid is not clear and has not been documented **(Kumari *et al.*, 2002)**. However, the inhibition of cholinesterase induced by test chemical is associated with the triazophos intoxication and it can be concluded that

triazophos (organophosphate insecticide) potentiate the toxicological effect of the deltamethrin.

Evaluation of haematological changes in laboratory animals exposed to environmental chemicals is important in the overall assessment of the risk and hazards. The effect of chemicals results in the destruction red blood cells or the inhibition of the haemopoiesis. Some agents stimulate hemopoiesis and other effect the function of blood cells **(Luind, 2000). Gupta *et al.*, (2001)** suggested that decreased hematological values may be attributed to the nutritional deficiency, during hepatotoxicity or due the inappetance. Present investigation was planned to evaluate the haematojgocal changes in rats treated with deltamethrin 1% + triazophos 35% EC.

There was a significant decrease in total erythrocyte count in rats of high dose group. No significant variation in total erythrocyte count was observed by **Bhelonde and Ghosh (2004)** following the exposure of a synthetic pyrethroids fenpropathrin in rats. Significant reduction in total erythrocyte count was found at 48 mg/kg body weight in rats treated with pendimethalin in a chronic exposure **(Ayub Shah *et al.*, 1998).** Reduction in haemoglobin content is due to physiological dysfunctioning of haemipoitic system, which may be due to decrease rate of erythrocyte synthesis and destruction of erythrocytes directly **(Siddiqui *et al.*, 1987).** Rats treated with test chemical revealed a dose dependent decline in haemoglobin and haematocrit value which is correlated with a decreased RBC count, which in turn might be due to the effect of the pesticides on blood forming organs suggesting anemia. Similar observation was made by **Rahman *et al.*, (1990).** Anaemia may occur as a primary clinical feature or secondary primary toxicity (i.e. nephrotoxicity or hepatotoxicity). All causes of anemia including hemolysis, haemorhages, blood loss, and decreased red blood cell production have been associated with the chemical exposure. R.B.C may be affected singly or in conjugation with primary or secondary affects on platelets and leukocytes **(Gossett, 2000).** The test material causing lower body weight will also affect the erythrocyte mass **(Hall, 1992). Bhelonde and Ghosh (2004)** reported a significant decrease in Hb and PCV with the exposure to synthetic pyrethroid (fenpropathrin) in rats. Toxicity evaluation of pendimethalin in rats (15 weeks exposure) revealed reduction in haemoglobin level **(Ayub Shah *et al.*, 1998). Dhembare and Pondhe, 2000** reported decrease in RBC, Hb and PCV values in fish following seven days treatment with phosphamidon, monocrotophos (organoposphate) and fenevalerate (synthetic pyrethroid).

The erythrocyte indices are calculated from haemoglobin concentration, packed cell volume and total erythrocyte count, which gives quantitative information about the red blood cells. Female rats exposed to the test substance revealed dose related decrease in the values of

MCH. Also observed was significant (p≤0 05) decrease MCHC value. This can be correlated with the decrease in the erythrocyte count and haemoglobin. Similar observation was made by **Choudhary and Joshi (2002)** in rats treated with endosulfan. Similarly, decrease in MCH in rat was reported by **Bhelonde and Ghosh (2004)** following fenpropathrin intoxication. A subacute toxicity study of deltamethrin (3, 6 and 12 mg/kg body weight) in rats did not reveal any significant difference among the control and treated groups indicating lack of adverse effect on haemopoitic organ at above dose levels **(Shiva Kumar *et. al.*, 2002). Malone and chester (1970)** also reported that bioresmethrin at 1000 mg/kg for 14 days did not alter any of the haematological parameters. **Hend and Butterwerth (1996)** reported no significant changes in RBC value in rats treated with cypermethrin at 1600 mg/kg.

Dose dependent increase in total leukocyte count (WBC) was observed in female rats treated with deltamethrin triazophos suggesting induction of some pathology as reported in lindane treated birds **(Mandal *et al.*, 1986)** and/or also may be due to effect on bone marrow **(Rahman,1996).** Total leukocytes count increased during pregnancy. It rises soon after delivery and then gradually returns to normal condition. A subchronic study of fenpropathrin (5.916mg/kg body weight) in rat did not reveal significant variation in total leukocyte count **(Bhelonde and Ghosh, 2004).** Slight reduction in WBC count was reported in a chronic toxicity study of pendimethain in rats **(Ayub Shah *et al.*, 1998)**

Determination of platelet count and clotting time are important tests for the investigation of bleeding disorder. Platelets (thrombocytes) contain ADP, various clotting factor and microtubules made up of thrombosthenin, which help the platelets to retract. The Main function of the thrombocytes is to assist haemostasis by aggregation, retraction and helping in coagulation. Decrease platelet production is most frequently associated with generalized suppression of hemopoiesis i.e. aplastic anemia **(Weiss, 2000).** Thrombocytopenia (decreased platelet count) occurred in various anaemias, supersplenism, and acute leukemia and in immune thrombocytopenia. Decreased platelet count is often associated with the prolonged bleeding and poor retraction **(Godkar, 1994).** A dose dependent decrease in platelet count and increase in clotting time was observed in rats due to exposure of deltamethrin 1% + triazophos 35% EC. The observed decrease in platelet count might be due to poor clot retraction whereas dose dependent significant increase in clotting time observed in present study correlated with the decrease in platelet count.

The liver is the largest glands in the body and is characterised by a multiplicity of complex functions, which includes excretion, secretion, storage detoxification, metabolism etc. Liver is the most important organ in the body and is known to play a major role in maintenance of haemostasis in the body. Therefore if the liver is damaged one or many functions would be

affected. In the present investigation, repeated dose oral exposure of deltamethrin and triazophos revealed changes in liver such as mottling and hepatomegaly (gross). Histopathological evaluation revealed lesion such as eosinophilic foci **(Figure 2.12)**, focal necrosis, hypertrophic foci and haemorrhagic area **(Figure 2.13)**. Haemorrhagic spots observed might be due to rupture of blood vessels caused by test substance. Eosinophilic foci consist of hepatocytes with a homogenous eosinophilic cytoplasm usually result from an increase in smooth endoplasmic reticulum. Hepatotocytes in eosinophilic foci tend to be larger than the surrounding hepatocytes and may cause compression of adjacent parenchyma **(Eustis *et al*, 1990)**. Hepatocytes can be dying by two different modes: necrosis and apoptosis. Necrosis is associated with swelling and influx of inflammatory cells. When necrosis occurs in hepatocytes, the associated plasma leakage can be detected biochemically by assaying liver cytosol derived enzymes including transminases (alanine aminotransferase and Aspartate aminotransferase) in plasma or serum which is simple method for screening population for potential liver necrosis caused by xenobiotics **(Moslen, 1996)**. Effect on biochemical profile investigated in rats treated with deltamethrin-triazophos showed significant elevation in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and that could be correlated with the damage of liver. **Bhelonde and Ghosh (2004)** had reported fatty changes, hydrophilic degeneration, sinusoids dilation, congestion and necrosis of hepatocytes at centrilobular region in liver of rats due to treatment of fenpropathrin. Deltamethrin induced granular degeneration and vacuolization in hepatic cells in rats **(Malpe *et al.*, 1996)**. Another investigation with synthetic pyrethroid (fenvalerate 35 % EC at dose concentration of 0.004%) revealed various cellular and sub cellular changes in the liver of squirrel, *Funambulus pennanti* **(Tripathi *et al.*, 2001)**. Histophothological examination of mice treated with chlorpyriphos 40% EC ($1/10^{th}$ of LD_{50}) for 10 days revealed congestion and degenerative changes of liver **(Revathi and Sunitha, 2000)**. Evaluation of ultrastructural changes in liver in rats treated with paralidoxima following acute organophosphate poisoning resulted in serious changes in hepatocytes and organelles which were found to be reversible with the treatment of paralidoxima **(Satar *et al.*, 2004)**.

The kidney is particularly important in studying toxicity because metabolites are eliminated primarily through the urinary tract. In addition the kidney is liable to quick exposure of circulating toxic solutes in the renal tubular cells or in the tubular lumen **(Greaves, 1995)**. The alteration in ALP activity and protein suggests renal damage and altered metabolic activity **(Gowenlock 1995)**. The Nephritic syndrome, which is almost always the result of glomerular injury, is characterized by hypercholesteremia **(Hall, 1992; Godkar, 1994)**. Rats with marked nephropathy may have hyper cholesterolemia or nephritic syndrome. **(Montgomery and Seely, 1990)**. In the present study, Kidney damage was visualized by

change in histological picture. Kidney showed nephropathic lesions such as dilation of renal pelvis, atrophy of renal papilla, transitional epithelial cell hyperplasia, and nephritic hyperplasia of pelvis **(Figure: 2.14 – 2.18)**. Hyperplasia of renal pelvis epithelium (urothelium) may occur spontaneously or it may be related to chemical treatment. Urothelial hyperplasia is commonly seen in the renal pelvis with severe spontaneous nephropathy. Treatment related hyperplasia in the transitional epithelium has been related to papillary necrosis, mineralization or calculi formation and chemically exacerbated nephropathy **(Montgomery and Seely, 1990).** Focus of mononuclear cells (MNC) aggregae by the side of pelvis with normal transitional epithelial cell lining of pelvis was recorded in the present study. Interstitial mononuclear cell infiltrates are usually associated with nephropathy. **Montgomery, and Seely (1990)** also suggested that the lymphocytic infiltrates are occasionally found around the arcuate arteries near the corticomedullary junction and beneath the transitional epithelium. Hypercholesterolemia and increase in urea nitrogen observed in the test article treated rats may be correlated with the kidney damage. **Bhelonde and Ghosh (2004)** reported tubular degeneration and necrosis in rat kidney following single exposure of fenpropathrin. Histopathological examination of mice treated with chlorpyriphos 40% EC ($1/10^{th}$ of LD_{50}) for 10 days revealed hyperemia in kidney **(Revathi and Sunitha, 2000). Tripathi *et al.*, (2001)** observed cellular and sub cellular changes in kidney of squirrel treated with synthetic pyrethroid fenvalerate 35 % EC at 0.004% dose concentration. A chronic toxicity study with synthetic pyrethroids, organophosphate and chlorinated hydrocarbon in broiler chicks revealed an increase in alkaline phosphatase and decrease in serum total protein as a result of pathophysiology **(Garg *et. al.*, 2004).**

The results of present finding revealed that the exposure of deltamethrin 1% + triazophos 35% EC at the dose level of 30 mg/kg body weight produces mild to moderate changes in the haemopoietic system. The currently observed, decline in erythrocyte count, hemoglobin and hematocrit with effect on total leukocyte count suggest the possibility of suppression of the haemopoietic activity as seen in most toxicity study using organophosphorus compound. Deltamethrin 1% + triazophos 35% EC is also able to induce hepatic changes and nephropathy. Moreover, all these changes are dose dependent and also happening at much lower dose indicating a possible potentiation of toxicity due to the combination of organophosphorus with pyrethroids.

TABLE 2.1 Biochemical parameter of male rats

Parameter	Group Number and Dose (mg/kg body weight)			
	G1 (0)	G2 (10)	G3 (20)	G4 (30)
GLU (mg/dl)	103 40 ± 0 46[@]	119 86 ± 0 77**↑	134 39 ± 1.35**↑	139.94 ± 1.28**↑
ALT (IU/L)	62.60 ± 0 46	71.18 ± 0 76**↑	74.60 ± 0.96**↑	78.95 ± 0.67**↑
AST (IU/L)	163.28 ± 3.42	169 43 ± 2.98	174 98 ± 3.36	177 30 ± 3 35*↑
BUN (mg/dl)	13.50 ± 0 28	16.20 ± 0.44**↑	19 00 ± 0 28**↑	21 38 ± 0.50**↑
T. PRO (g/dl)	7.22 ± 0 02	7 45 ± 0 02**↑	7.69 ± 0.03**↑	7 75 ± 0.06**↑
ALB (g/dl)	3.76 ± 0 03	3 79 ± 0.02	3.84 ± 0 02	3.87 ± 0.03*↑
Ca^{+} (mg/dl)	10.00 ± 0.14	10 00 ± 0 00	9.80 ± 0.18	9 99 ± 0.10
CHO (mg/dl)	63 71 ± 1.16	69 12 ± 1.84*↑	74.01 ± 1 62**↑	80.04 ± 1.02**↑
NA^{+} (mmol/l)	127.34 ± 1.35	131 92 ± 1.30*↑	135.86 ± 1.42**↑	138 84 ± 0.85**↑
K^{+}(mmol/l)	4.46 ± 0 09	4 64 ± 0 04	4 87 ± 0 11**↑	5 00 ± 0 04**↑
Cl (mmol/l)	113 50 ± 1.17	108.90 ± 0.86*↓	106.50 ± 0.47**↓	104.64 ± 1.88**↓
ALP (IU/L)	169.63 ± 5.49	187 59 ± 7.21↑	196 40 ± 7.84*↑	211.44 ± 7.18**↑
ChE (IU/L)	203.46 ± 9 67	115 06 ± 7.22**↓	97.14 ± 2.41**↓	72.40 ± 1.37**↓

[@] Mean ± SE, * $p \leq 0.05$, ** $p \leq 0$ 05

TABLE 2.2 Biochemical parameters of female rats

Parameter	Group Number and Dose (mg/kg body weight)			
	G1 (0)	G2 (10)	G3 (20)	G4 (30)
GLU (mg/dl)	107 68 ± 0 59[@]	116 46 ± 1.64*↑	120.80 ± 2.83**↑	126.46 ± 2.84**↑
ALT (IU/L)	59.79 ± 2.17	64.74 ± 3 04	76.21 ± 2.32**↑	80.70 ± 3 56**↑
AST (IU/L)	173 43 ± 2 93	180 22 ± 3.78	188.68 ± 3.04*↑	203.00 ± 3.56**↑
BUN (mg/dl)	17 80 ± 0.52	22 40 ± 0 88**↑	29 80 ± 0 87**↑	32.38 ± 0.92**↑
T PRO (g/dl)	7.12 ± 0 13	7 24 ± 0.06	7.44 ± 0.10	7.98 ± 0.29**↑
ALB (g/dl)	3.70 ± 0 06	3 74 ± 0 04	3.78 ± 0 03	3 82 ± 0.04
Ca^{2+} (mg/dl)	9.80 ± 0 33	9 80 ± 0.08	9 86 ± 0 09	9.90 ± 0.11
CHO (mg/dl)	66 52 ± 2.19	69.12 ± 3 26	74 86 ± 2.95	80.66 ± 3.23**↑
Na^{+} (mmol/l)	128.36 ± 4.08	130 94 ± 1.42	135.96 ± 1.31	137.92 ± 2.62*↑
K^{+}(mmol/l)	4 24 ± 0 09	4.40 ± 0 10	4 59 ± 0 09*	4.70 ± 0.06**↑
Cl (mmol/l)	114.44 ± 0.47	103 30 ± 2.05*↓	98.10 ± 2 43**↓	91 60 ± 4.14**↓
ALP (IU/L)	183.76 ± 5.81	199 67 ± 6 22	220.74 ± 8 13**↑	227.74 ± 4.64**↑
ChE (IU/L)	208.60 ± 8 03	129.48 ± 4 66**↓	93 40 ± 3.56**↓	77.40 ± 12.69**↓

[@] Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.05$

TABLE 2.3 Haematological parameters of male rats

Parameter	Group Number and Dose (mg/kg body weight)			
	G1 (0)	G2 (10)	G3 (20)	G4 (30)
WBC ($x10^3$/µl)	12.42 ± 0.63[@]	12.14 ± 0.73	11.98 ± 0.67	12.15 ± 0.67
RBC ($x10^6$/µl)	8.40 ± 0.03	8.30± 0.05	7.94 ± 0.04**↓	7.53 ± 0.05**↓
Hb (g/dl)	15.80 ± 0.16	15.66 ± 0.20	15.40 ± 0.15	14.93 ± 0.27*↓
HCT (%)	39.14 ± 0.33	38.78 ± 0.22	38.16 ± 0.45	37.33 ± 0.38**↓
MCV (fl)	46.74 ± 0.72	46 78 ± 0 75	46.42 ± 0.39	47.40 ± 0.63
MCH (pg)	18.86 ± 0.39	18.78 ± 0.38	18.84 ± 0.30	18.85 ± 0.31
MCHC %	40.32 ± 0.31	40.14 ± 0.26	41.18 ± 0.27	37.13 ± 0.30**↓
PLT ($x10^3$/µl)	986.40 ± 52.27	970 60 ± 22.77	958.20 ± 22.92	935.25 ± 81.13
CT (second)	80.60 ± 4.63	86.20 ± 2.25	91.20 ± 4.68	105.00 ± 8.66*↑

[@] Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.01$

TABLE 2.4 Haematological parameters of female rats

Parameter	Group Number and Dose (mg/kg body weight)			
	G1 (0)	G2 (10)	G3 (20)	G4 (30)
WBC ($x10^3$/µl)	5.46 ± 0.64[@]	6.90 ± 0.92	7.54 ± 0.91	8.2 ± 0.99
RBC ($x10^6$/µl)	8.29 ± 0.07	8.12 ± 0.06	8.05 ± 0.08	7.62 ± 0.06**↓
Hb (g/dl)	15.48 ± 0.17	15.16 ± 0.21	14.94 ± 0.19	14.76 ± 0.28
HCT (%)	38.64 ± 0.36	37.14 ± 0.30*↓	36.96 ± 0.31**↓	36.54 ± 0.37**↓
MCV (fl)	48.72 ± 1.03	48.50 ± 0.91	48.36 ± 0.63	48.60 ± 0.47
MCH (pg)	20.20 ± 0.40	20.16 ± 0.24	19.98 ± 0.28	19.44 ± 0.28
MCHC %	40.28 ± 0.41	39.78 ± 0.45	40.42 ± 0.38	38.72 ± 0.43*↓
PLT ($x10^3$/µl)	960.60 ± 44.71	956 20 ± 37.78	923.80 ± 16.13	918 80 ± 22.50
CT (second)	72.00 ± 12.00	78.00 ± 7.35	102.00 ± 12.00	108.00 ± 7.35

[@] Mean ± SE, * $p \leq 0.05$, ** $p \leq 0$ 01

Figure 2 1 Serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) Activity in male and female rats subjected to combination pesticide at various doses

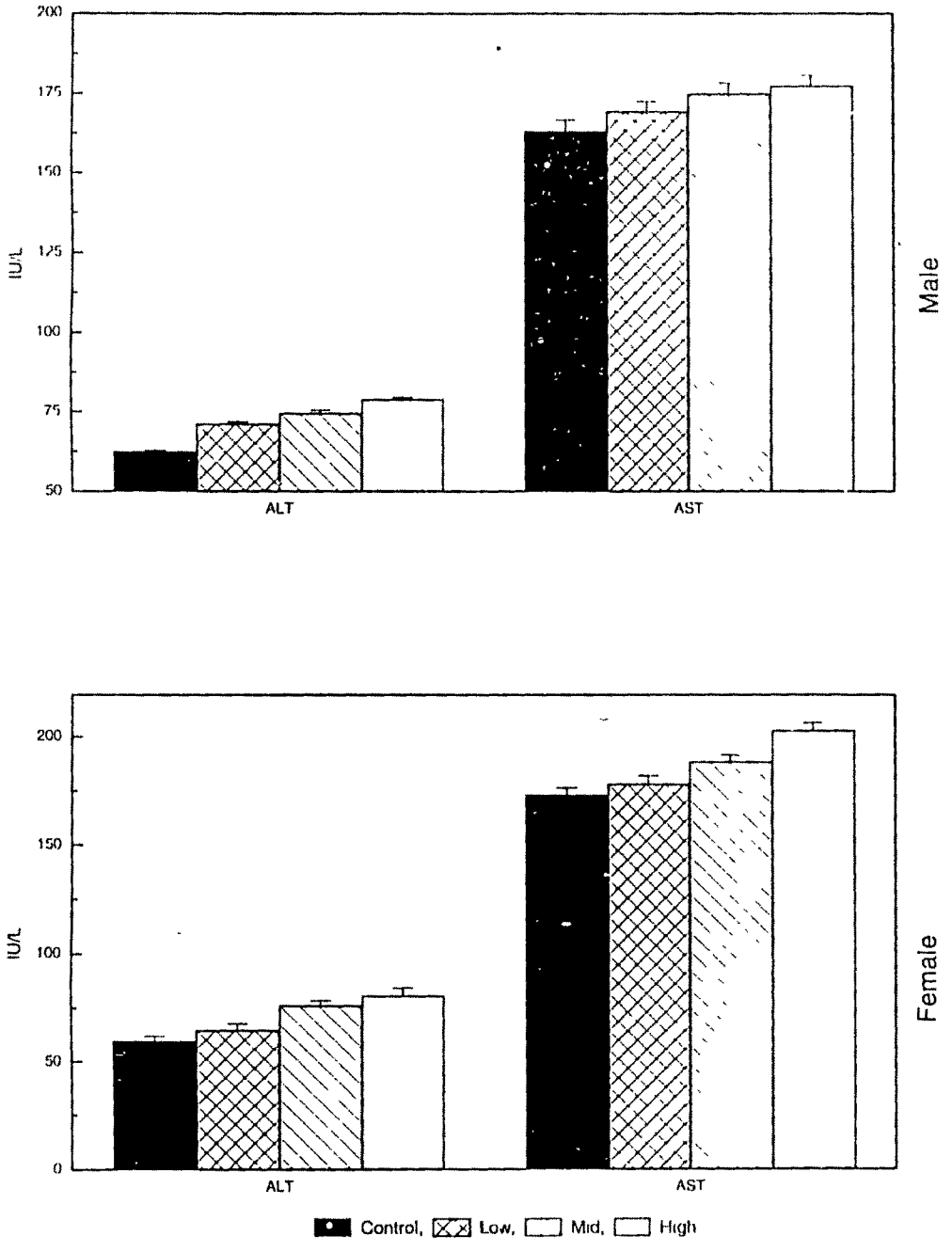


Figure 2.2 Alkaline Phosphatase (ALP) and Cholinesterase (ChE) activity in male and female rats subjected to combination pesticide at various doses

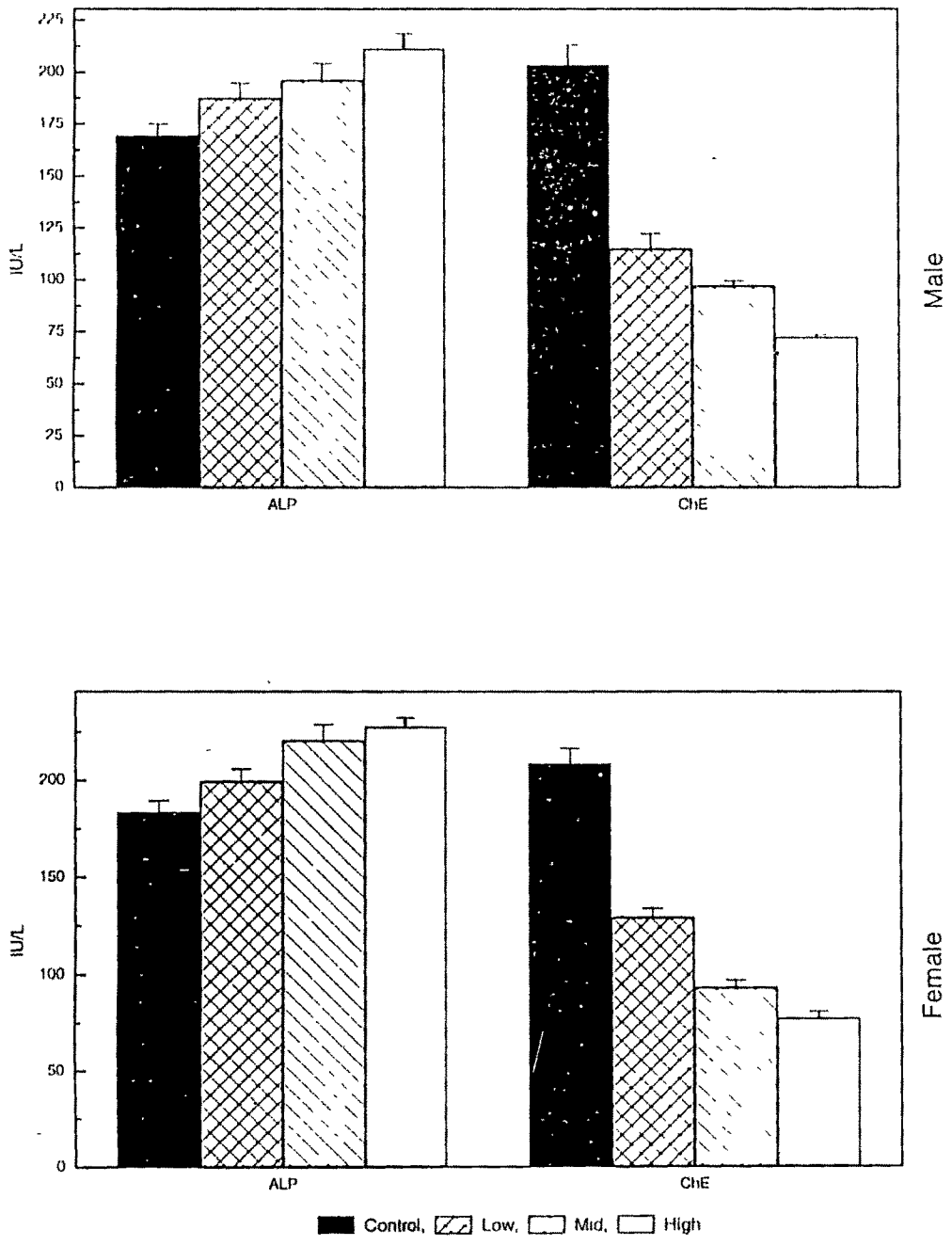


Figure 2 3 Serum Glucose and BUN levels Activity in male and female rats subjected to combination pesticide at various doses

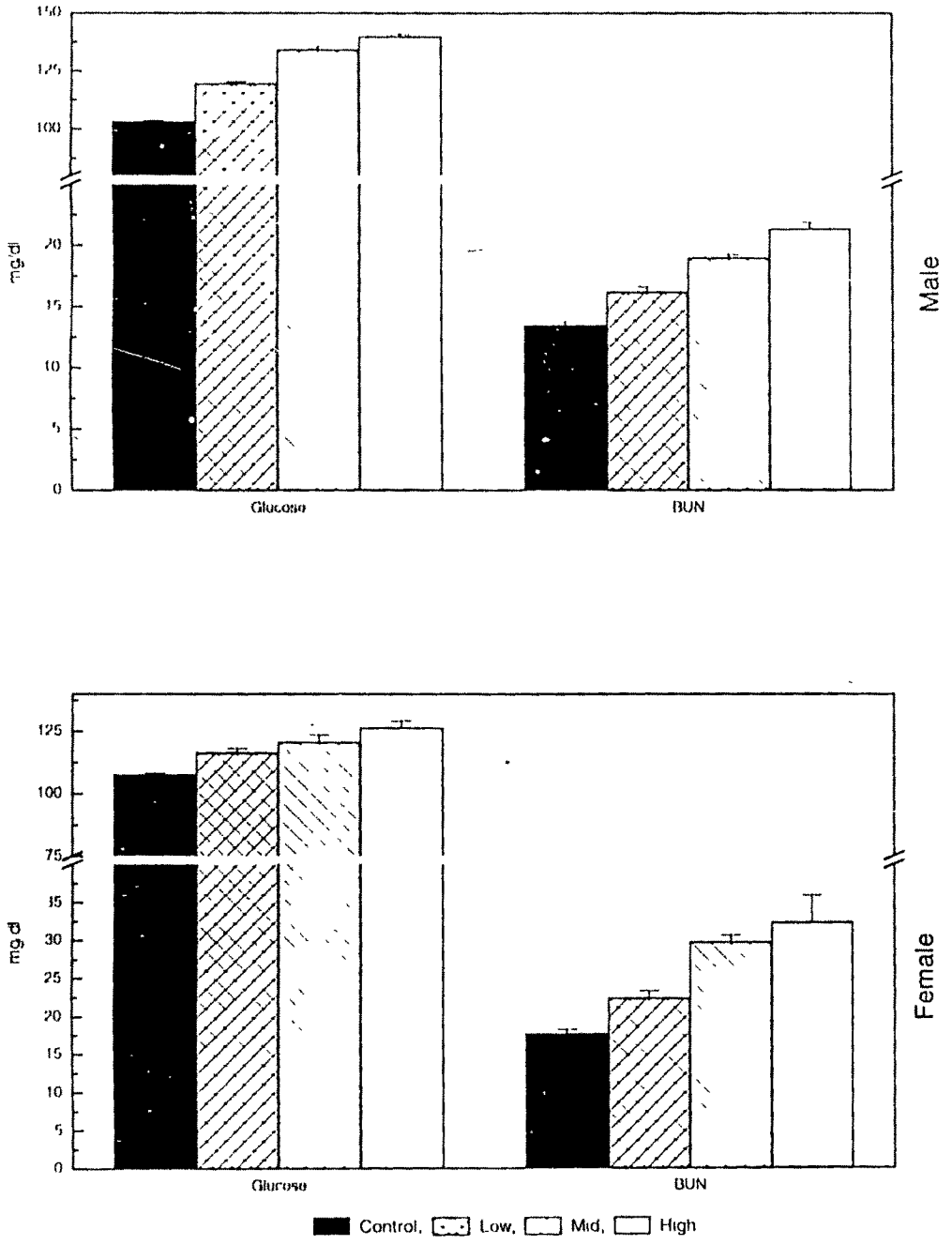


Figure 2 4 Serum cholostorol (CHO) , Albumin (Alb) and total portein (T Pro) levels in male and female rats subjected to combination pesticide at various doses

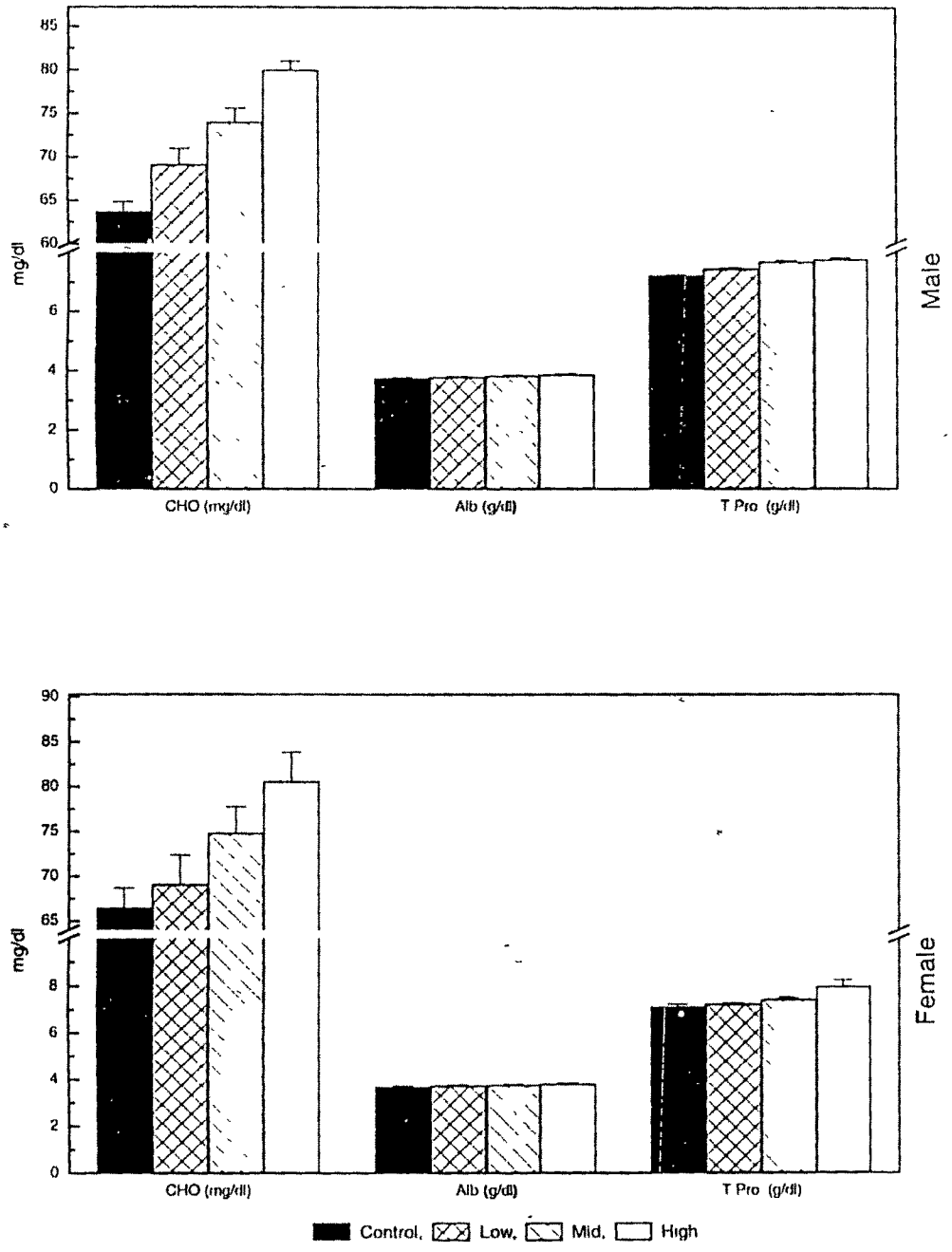


Figure 2.5 Serum Sodium(Na^+) and Chloride(Cl^-) levels in male and female rats Subjected to combination pesticide at various doses

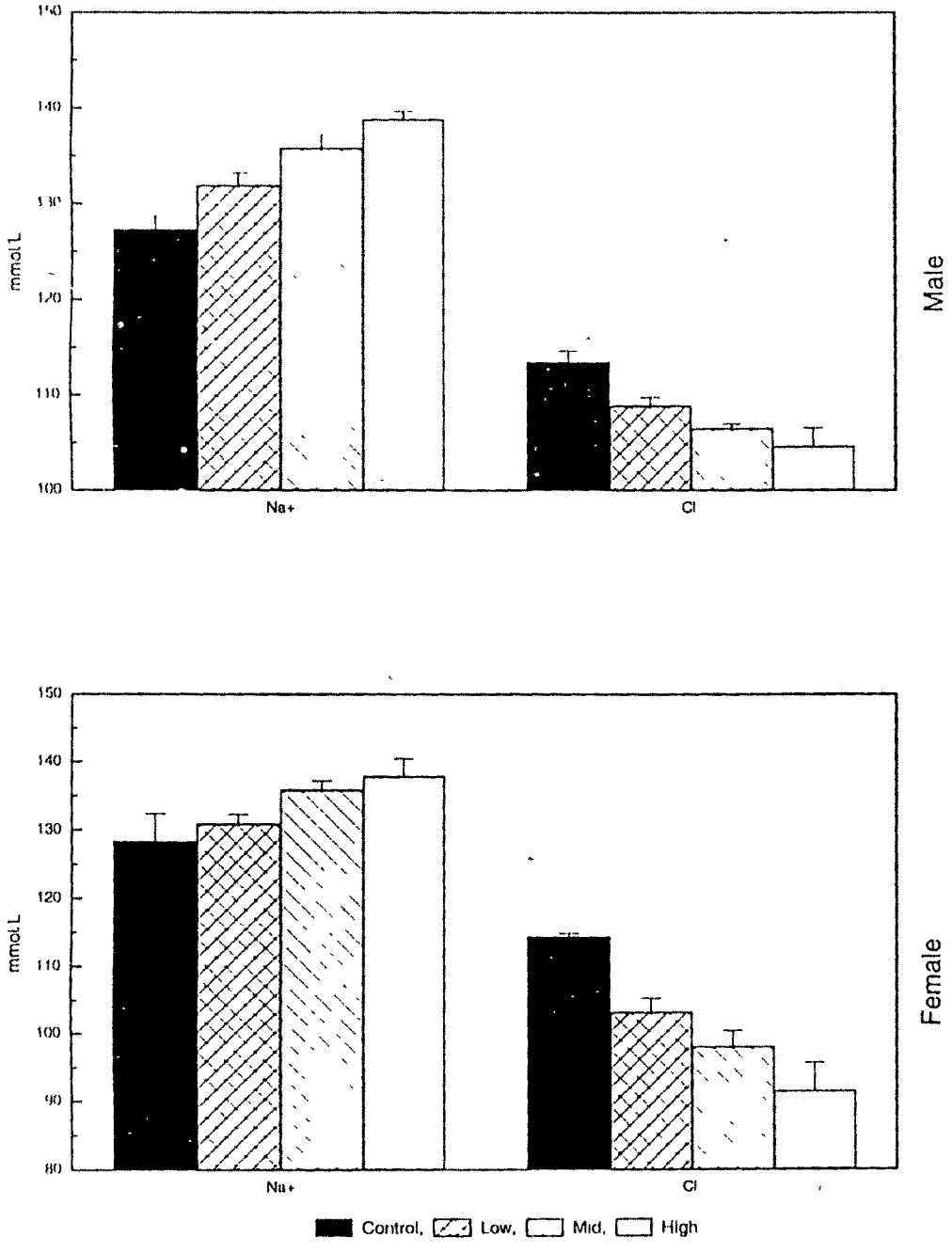


Figure 2.6 Serum Calcium (Ca^{2+}) and Potassium (K^{+}) levels in male and female rats subjected to combination pesticide at various doses

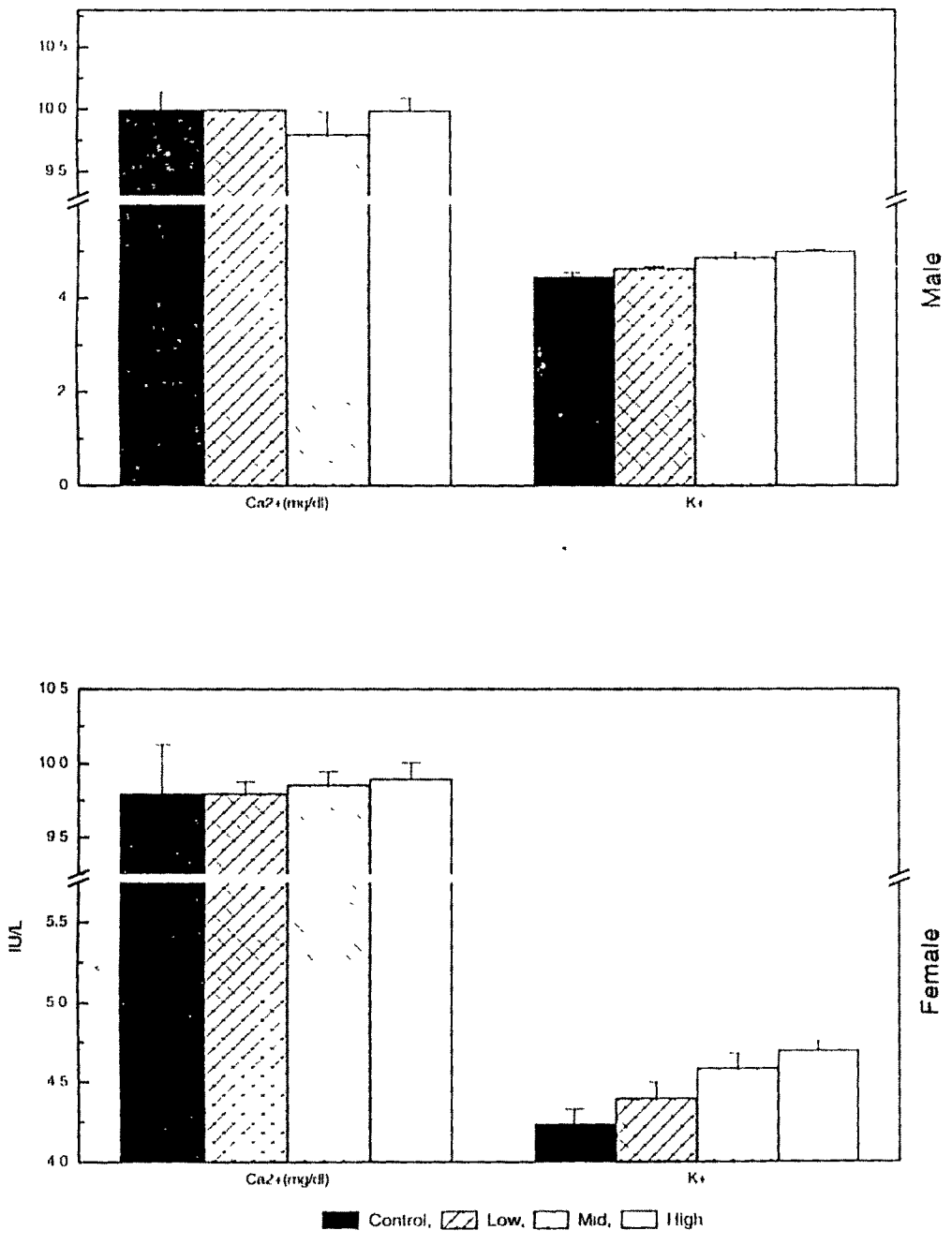


Figure 2 7 Total white blood corpuscles (WBC) and red blood corpuscles (RBC) in male and female rats subjected to combination pesticide at various doses

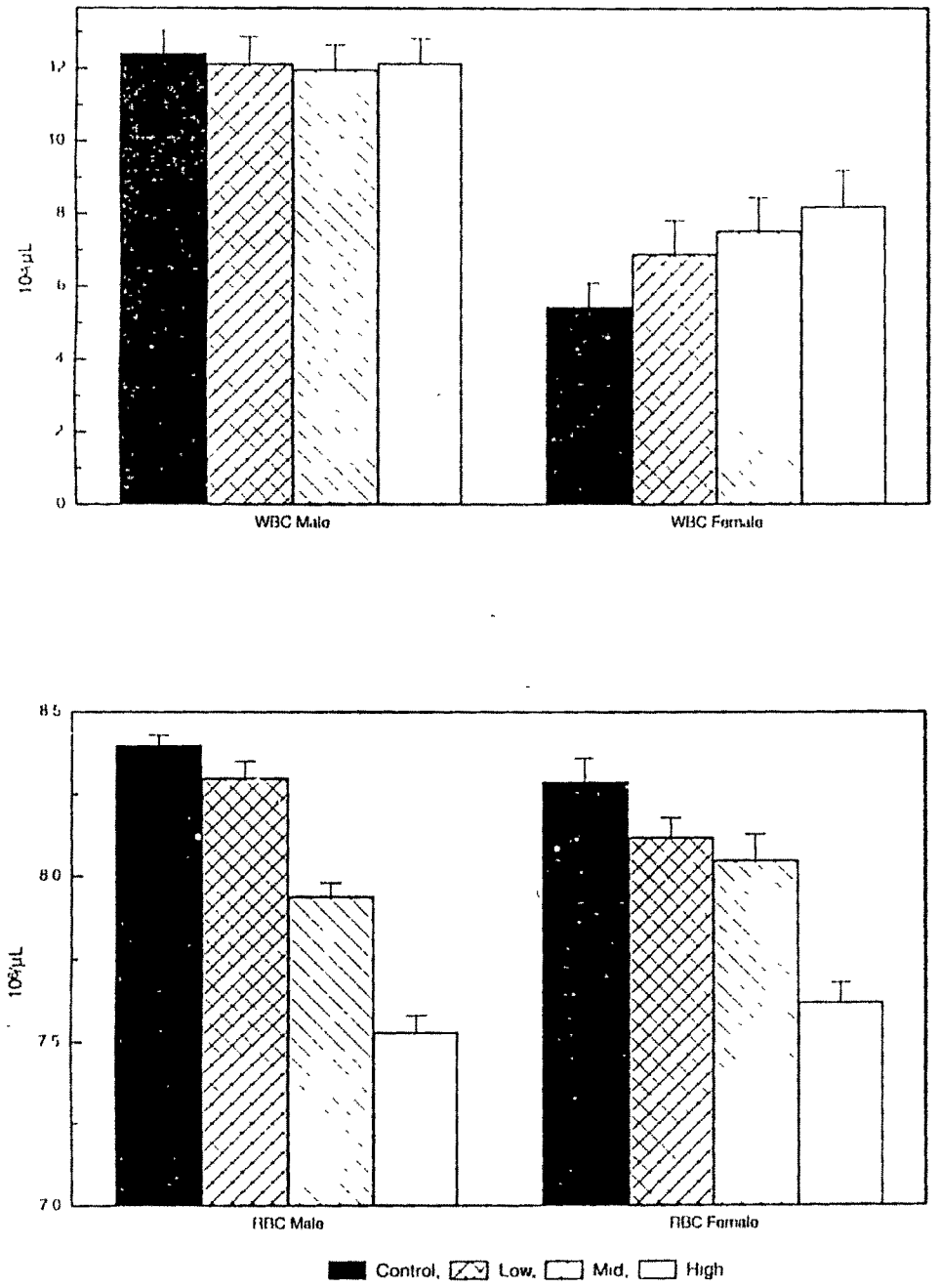


Figure 2.8 Haemoglobin content (Hb) and Haematocrit value (Hct) in male and female rats subjected to combination pesticide at various doses

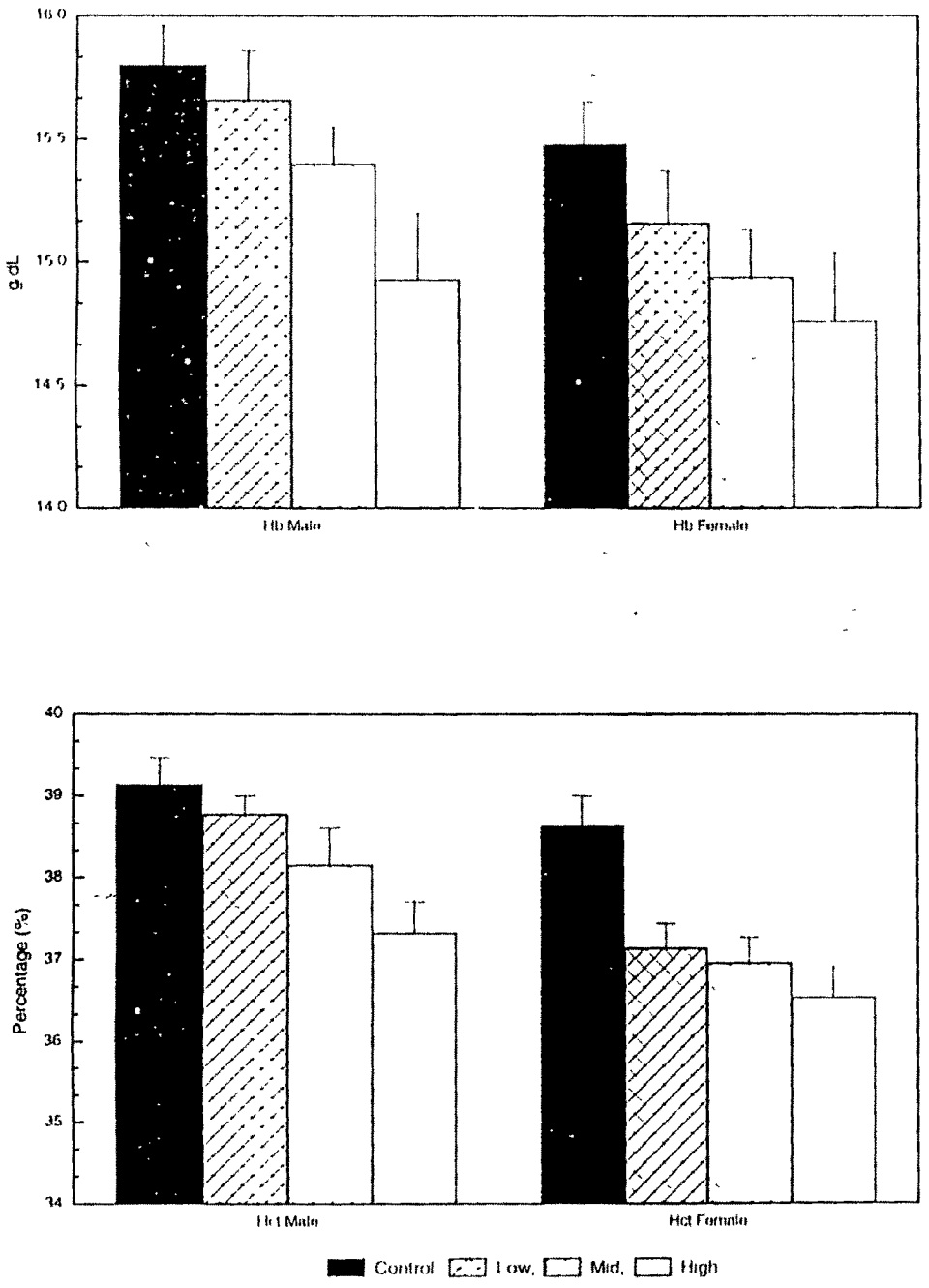


Figure 2 9 Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin (MCH) in male and female rats subjected to combination pesticide at various doses

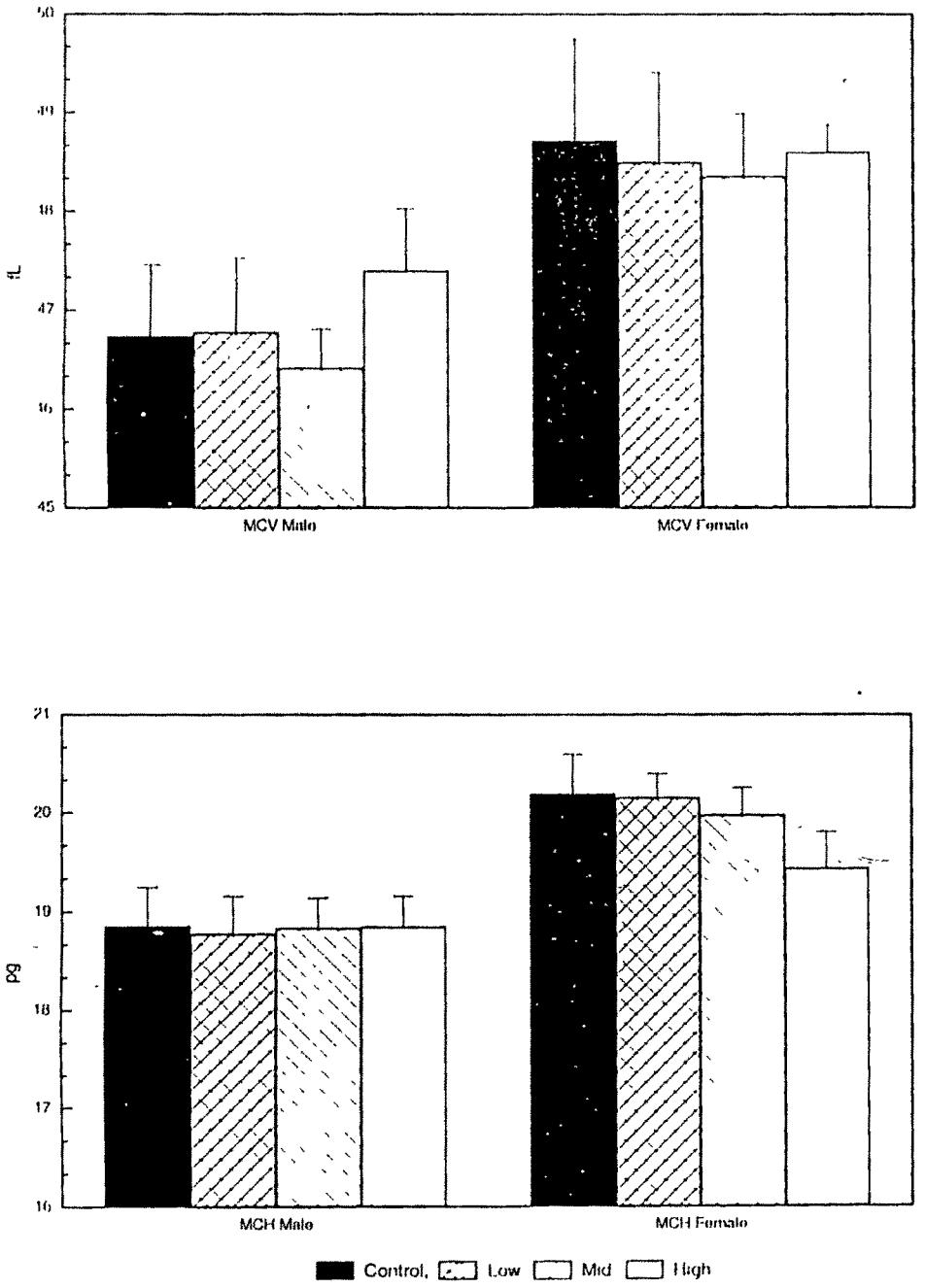
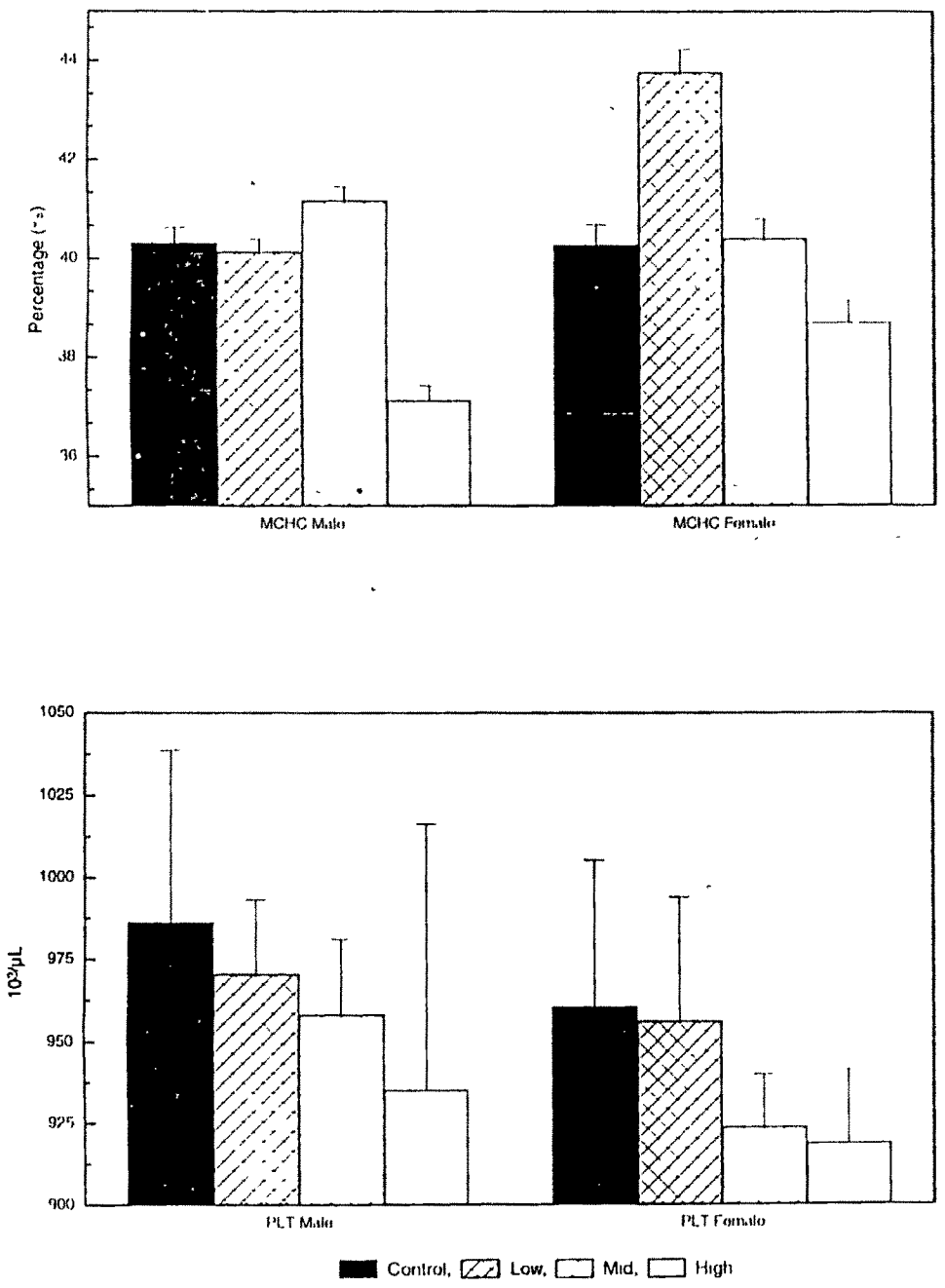


Figure 2.10 Mean corpuscular haemoglobin concentration (MCHC) and platelet number (PLT) in male and female rats subjected to combination pesticide at various doses



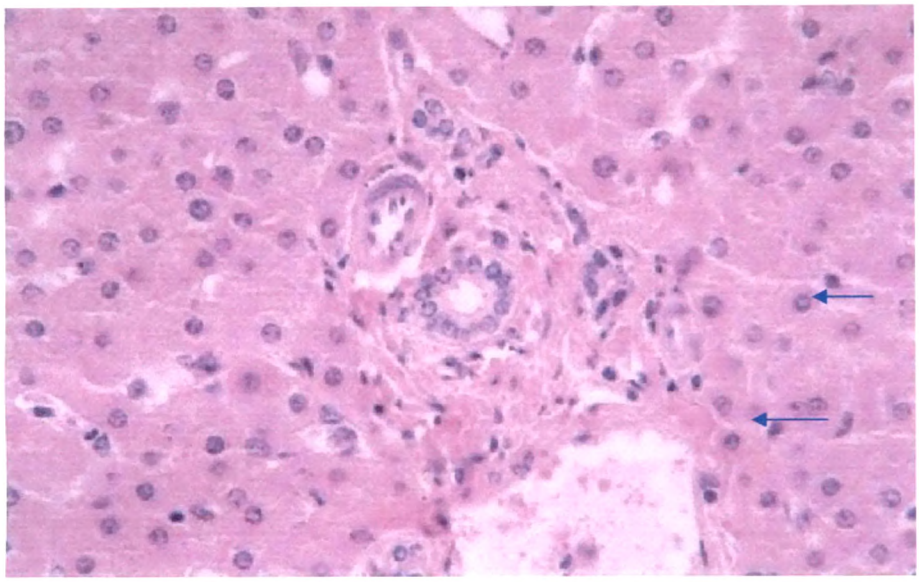


FIGURE 2.11. Normal liver showing normal hepatocyte(←) x 40

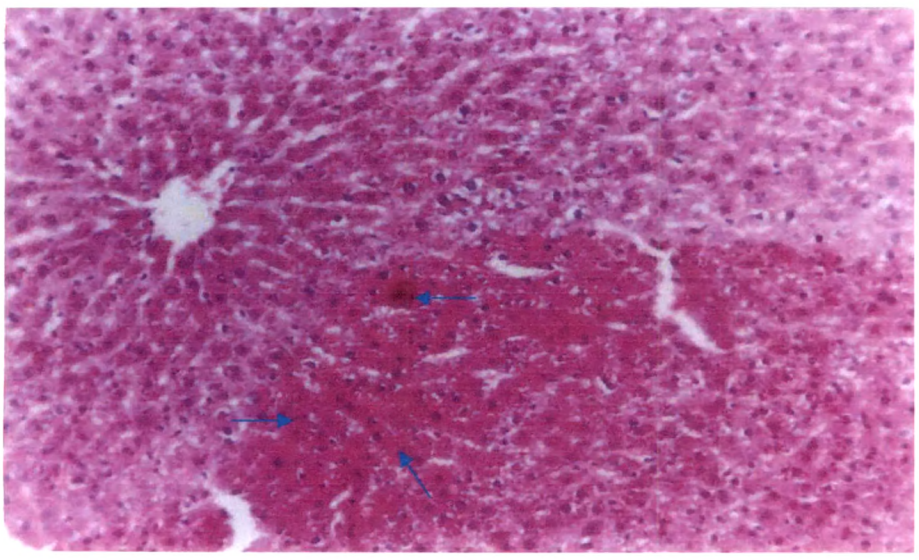


FIGURE 2.12 Liver showing Eosinophilic foci (←) X 10

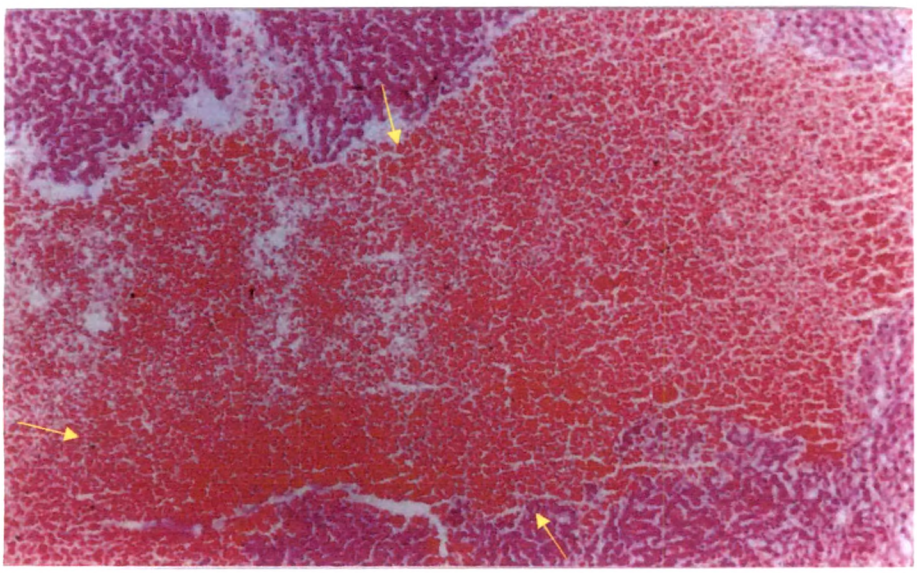


FIGURE 2.13. Liver showing diffuse haemorrhagic area(←) X 4

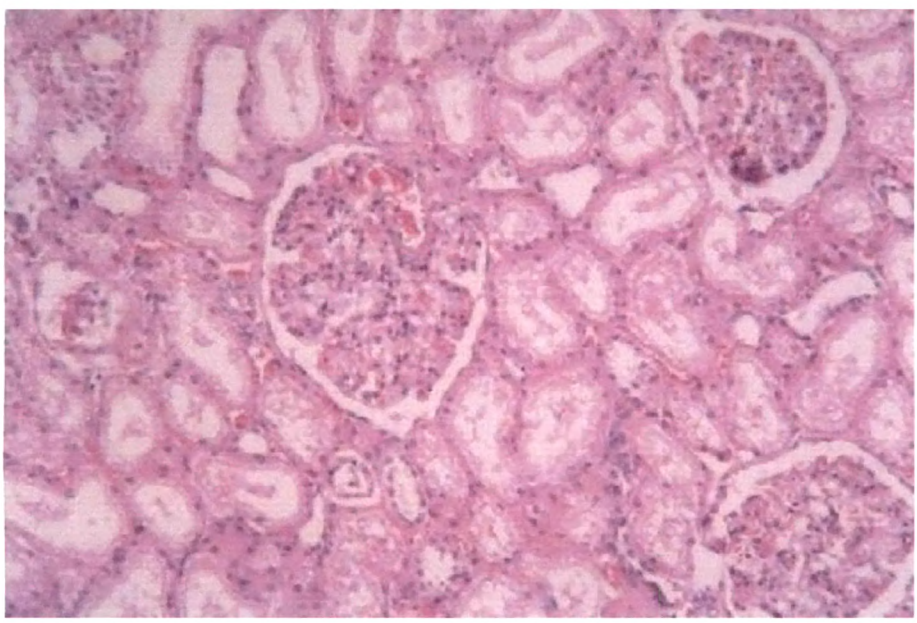


FIGURE 2.14 Normal Kidney showing glomerulus and tubules X 4

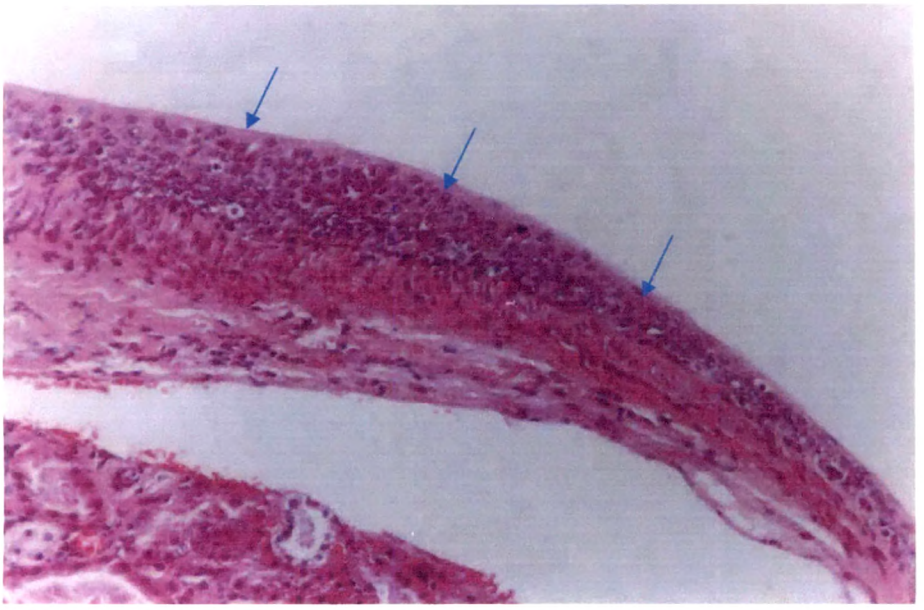


FIGURE 2.15. Kidney showing transitional epithelial cell hyperplasia X 10 (←)

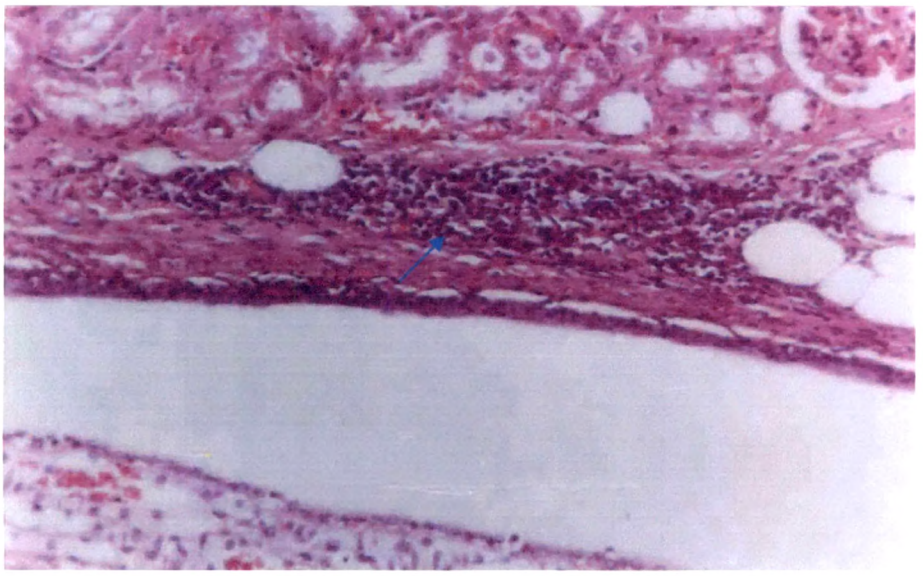


FIGURE 2.16. Kidney showing focus of mononuclear cells aggregate by the side of pelvis with normal transitional epithelial cell lining of pelvis X 10 (←)

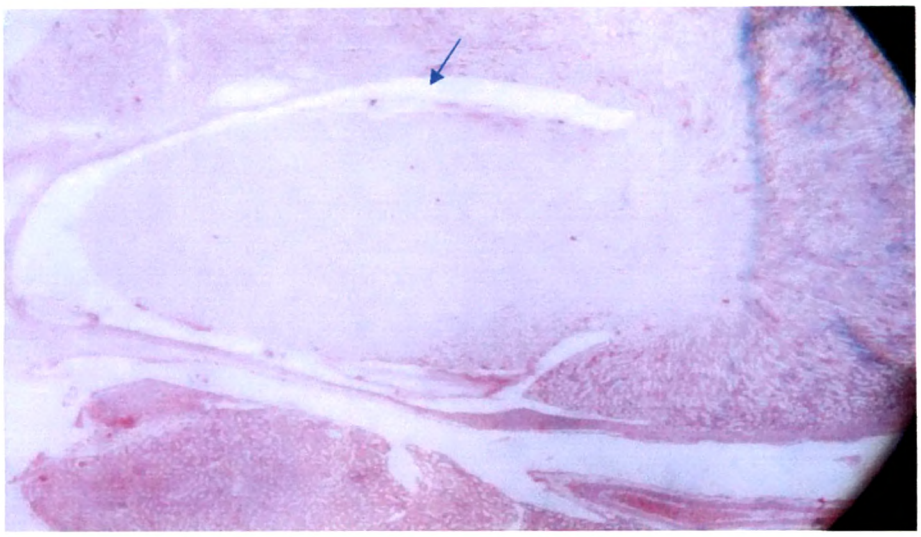


FIGURE 2.17 Kidney showing dilated pelvis X 10 (←)

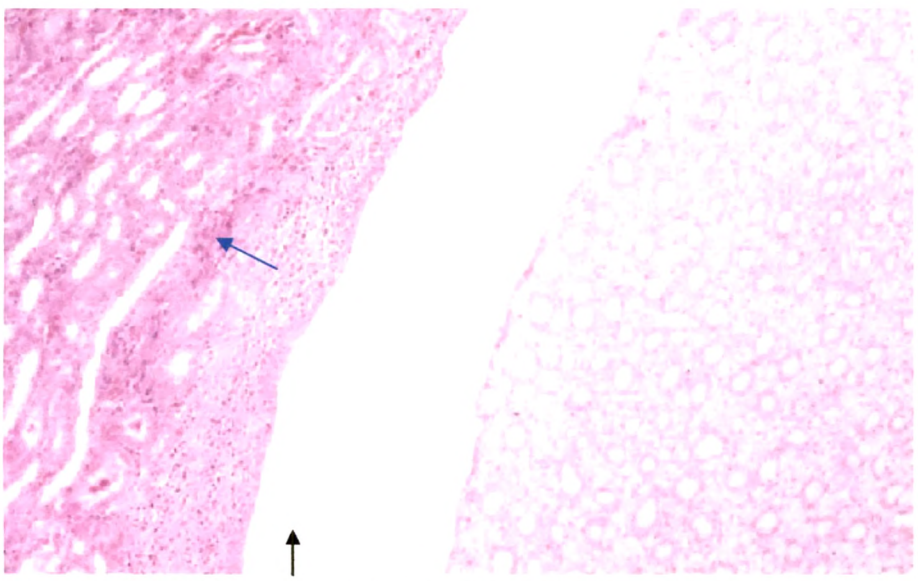


FIGURE 2.18 Kidney showing focal pyelitis (←) and dilated pelvis (⟵) X 40