GASTROINTESTINAL AND NEUROTOXIC EFFECTS OF CYPERMETHRIN IN RATS

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DECLARATION

I hereby declare that this thesis entitled **"GASTROINTESTINAL AND NEUROTOXIC EFFECTS OF CYPERMETHRIN 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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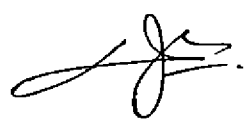
Certified that the thesis entitled **"GASTROINTESTINAL AND NEUROTOXIC EFFECTS OF CYPERMETHRIN IN RATS"** is a record of research work done independently by **Dr. Remya R. Nair** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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Remya R. Nair

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Pesticides play a versatile role in the control of insects/ pests and rodents in modern agriculture. The intensive and stupendous use of pesticides has greatly aided in enhancing crop production and providing protection to man and livestock from diseases. Widespread use of pesticides in agriculture and livestock production has posed potential health hazard not only to livestock and wildlife but also to fishes, birds, mammals and even human beings (Patel *et al.*, 2000). Toxic hazards to livestock are of common occurrence, particularly in developing countries, resulting in decrease in the productivity and their physiological functions.

Most of the insecticides in current use fall under broad groups like Chlorinated Hydrocarbons, Organophosphorus Compounds, Carbamates and synthetic pyrethroids. Several ecological and public health problems also surfaced with the indiscriminate and unscientific application of pesticides. Reports of the presence of pesticides in water and food materials have aroused great concern among the health scientists and regulatory agencies and thus led to studies on their toxic potential (Seth *et al.*, 2000). Organophosphorus and carbamate compounds are rapidly biodegraded in the environment whereas organochlorine chemicals are notorious for their persistence in the environment and accumulation in the food chain.

The synthetic pyrethroids constitute an unique group of insecticides having pyrethrin like structure with better performance characteristics and account for over 30% of insecticides used globally (Sayim *et al.*, 2005). Pyrethroids are modified derivatives of pyrethrins, the natural substances obtained from the flowers of *Chrysanthemum cineraiaefolium.* These pyrethroids are photostable with improved physical and chemical properties and greater biological activity as compared with pyrethrins. Pyrethroids, which rank among the most potent insecticides known, are widely used for field pest control, as household pesticide and as veterinary and

human pediculicides. The widespread use of these pesticides thus exposes the manufacturing workers, field applicators, the ecosystem and finally the public to the possible toxic effects of these insecticides.

The pyrethroids are potent neurotoxicants in both vertebrates and invertebrates, but acute toxicity in mammals is low (Giray *et al.*, 2001). The site of action of pyrethroids is in the biological membrane. In fact, the principal target site for pyrethroids is defined as the voltage dependent channels in the neuronal membrane. Neuroexcitatory symptoms of acute poisoning in vertebrates by pyrethroids are related to the ability of these insecticides to modify electrical activity in various parts of nervous system but repetitive nerve activity results from a prolongation of the sodium current during membrane excitation (Vijverberg and Bercken, 1990). On the basis of different behavioral, neurophysiological and biochemical profiles, two distinct classes of pyrethroids have been identified. Type I pyrethroids may cause mainly hyperexcitation and fine tremors while Type II pyrethroids produce a more complex syndrome including clonic seizures.

Cypermethrin, a recent synthetic pyrethroid is extensively used for plant protection and control of ectoparasites of domestic animals (Varshneya *et al.*, 1992). It is a Type II pyrethroid which is rapidly absorbed from the digestive tract and its excretion takes a quick course. Interestingly, cypermethrin persists in the air, on the walls and on the furniture for about three months after household treatment. In spite of its low toxicity, the persistence of pyrethroids in mammalian tissues may prove dangerous (Sayim *et al.*, 2005). Persistence of cypermethrin and its fatty acid conjugates in adipose tissue, brain and liver have been reported in rats. However, several authors having reviewed pyrethroid neurotoxicity concluded that pyrethroids do not tend to accumulate in the body and that their removal from nervous system as well as their excretion is rapid, even after repeated administrations (Cantalamessa, 1993). Several studies have demonstrated the hepatotoxic potential of cypermethrin

in rodents and its action as an immunosuppressant and neurotoxin in mammals as well as insects.

The present study is designed to evaluate the effects of cypermethrin in different doses in adult Sprague Dawley rats using gingely oil as a vehicle. A twenty one day repeated dose oral toxicity study is envisaged. Besides, biochemical and haematological alterations in rats will be assessed. The overall objective of the current study is to delineate the sub acute neurotoxic and gastrointestinal toxic potential of orally administered cypermethrin in adult male Sprague Dawley rats.

Review of Literature

2. REVIEW OF LITERATURE

Pesticides are the most widely used agrochemicals of toxicological importance. Pesticides consist of a large variety of chemical agents having diverse chemical structure and biological activities which are intentionally introduced into the environment for protection against pests of plants, animals and human importance. Pesticides helped in bringing about the green revolution and making the country self sufficient in food grains, pulses, vegetables, fruits etc. Moreover their use in animal husbandry and public health improves the health and productivity of animals and birds and the health of mankind. Pesticide include large group of chemicals such as insecticides, herbicides, rodenticides, weedicides etc.

Insecticides comprise different classes such as organochlorine, organophosphorus, carbamate and pyrethroid insecticides. Synthetic pyrethroids are one of the most widely used insecticides today, which possess potential insecticidal properties with low mammalian toxicity.

2.1 CYPERMETHRIN

Cypermethrin, (R, S) – α cyano- 3 phenoxy benzyl (1R, S) cis, trans- 3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate,belongs to Type II pyrethroid and possess α cyanogroup. It is photo stable and possesses high insecticidal activity. Cypermethrin, a synthetic pyrethroid insecticide, is a mixture of eight different isomers that can produce toxicological and ecological effects (Extoxnet, 1996). Alpha cypermethrin is a pyrethroid that acts predominantly on the central nervous system and high dosages have been found to induce tonic seizures in experimental animals.

2.2 TOXICOKINETICS

Pyrethroids do not accumulate in the body and their excretion is rather rapid even after repeated administration. Typically, 90% of the administered dose is excreted in urine and faeces within a week after treatment. Cypermethrin can be

absorbed into the rat skin to a significant extent. Occlusion of the skin surface does not appear to affect the percutaneous penetration of the chemical into the receptor fluid (Hotchkiss *et al.*, 1990). After oral administration, absorption of pyrethroids from the gut is incomplete with a tendency of these lipophilic molecules to remain in the organic solvent or oil used for oral administration (Sandhu and Brar, 2003). The pyrethroid hydrolysis is mostly carried out in liver by carboxyl esterase enzymes in liver microsomes (Stok *et al.*, 2004). The concentrations of malondialdehyde in tissues were observed to increase significantly in rats treated with cypermethrin. Vitamin E could modify the cypermethrin metabolism and play a protective role in cypermethrin mediated oxidative stress. The use of selenium prior to cypermethrin was observed to increase the malondialdehyde concentrations and antioxidant systems such as glutathione peroxidase and catalase activities and could alleviate oxidative stress induced by cypermethrin (Atessahin *et al.*, 2005). Studies in rats have shown that cypermethrin is rapidly metabolized by hydroxylation and cleavage, with over 99% being eliminated within hours. The remaining 1% becomes stored in the body fat and this portion is eliminated slowly, with a half life of 18 days for the cis-isomer and 3-4 days for the trans- isomer (Sayim *et al.*, 2005). Crow *et al.*, (2007) demonstrated extra hepatic esterolytic metabolism of specific pyrethroids in small intestine and serum of human and rat tissue. They reported that there is a difference in hydrolytic activity of various tissues against different pyrethroids.

2.3 MECHANISM OF ACTION

Pyrethroids are potent neurotoxic insecticides that disrupt normal nerve function by prolonging normal gating kinetics of voltage gated sodium channels of vertebrate and invertebrate nerve and vertebrate muscle (Smith and Soderlund, 1998). The α cyano containing or Type II pyrethroids extend the time constant for inactivation of sodium gate by hundredth of a millisecond to seconds producing a persistent depolarization and frequency dependent conduction block in sensory and motor axons and prolonged repetitive firing of sensory nerves and organs and muscle

fibres. In addition, cypermethrin tends to inhibit calcium and magnesium ATPase activities, the effect of which resulted in increased release of intracellular calcium levels accompanied by increased neurotransmitter release (Garg, 2006).

2.4 TOXICITY OF CYPERMETHRIN

2.4.1 Neurotoxic Effects

Cypermethrin increased cerebellar cyclic AMP at the earliest time measured, without changing cyclic GMP. An increase in the levels of glucose, lactate and ammonia was found in cerebellum of rats after intravenous administration of cypermethrin at a rate of 25 mg/kg body weight (Lock and Berry, 1981).

Tandon and Gupta (1990) opined that monoaminergic (especially norepinephrine and Gamma amino butyric acid) neurotransmitters could be involved in cypermethrin induced toxicity and that diazepam and Phenobarbital sodium could be of therapeutic value in the treatment of cypermethrin toxicity.

The neuroexcitatory symptoms of acute poisoning in vertebrates by pyrethroid are related to the ability of these insecticides to modify electrical activity by stereo selective and structure related interactions with voltage dependent sodium channels, the primary target sites of pyrethroids (Vijverberg and Bercken ,1990).

Luty *et al.*, (1998) observed neurotoxic property of cypermethrin by increased activity of acetylcholine esterase in cerebral cortex, cerebellum, striatum, hypothalamus and hippocampus of rats.

The pyrethroid insecticide acts by binding to a unique site in the voltage dependent sodium channels thus prolonging sodium currents leading to repetitive bursts of action potentials or by strong dependent nerve block. The pyrethroid binding site is intrinsic to sodium channel alpha subunit and it was demonstrated that

co expression of the rat brain II a alpha subunit with the rat beta subunit altered the apparent affinity of this site for pyrethroids(Smith and Soderlund, 1998).

Latuszynska *et al.*, (2001) concluded that the neurotoxic effect of a mixture of chlorpyriphos and cypermethrin in Wistar rats was not stronger than that caused by alpha cypermethrin alone.

The rat peripheral nerve tetradotoxin resistant voltage sensitive sodium channel was expressed in *Xenopus laevis* oocytes and the responses of expressed channels to pyrethroids , cismethrin and cypermethrin were assessed. Each pyrethroid produced two distinct modifications of sodium currents carried by SNS/PN3 channels; a sustained, slowly inactivating current evident during a depolarizing pulse and a prominent tail current following repolarisation (Smith and Soderlund, 2001).

Alpha cypermethrin potentiated pentobarbitone induced sleeping time and pentylene tetrazole induced convulsion concomitant with a decrease in GABA concentration in brain of rats was reported by Manna *et al.*, (2005a).

Deltamethrin significantly reduced the motor coordination, decreased onset of time and increased the sleeping time duration induced by pentobarbitone and decreased onset time and increased duration of convulsions induced by pentylenetetrazole in the study of neuropharmacological effects of deltamethrin in rats. They also observed a correlation between the effect of deltamethrin on the central GABA levels and its neuropharmacological levels (Manna *et al.*, 2006b).

2.4.2 Biochemical Effects

Elevation of blood glucose, lactate and ammonia in rats was reported by Lock and Berry, (1981), the intravenous administration of cypermethrin at a rate of 25 mg/kg body weight.

Feeding of albino rats with cypermethrin for six months produced elevation in the levels of serum lactate dehydrogenase, isocitrate dehydrogenase, and amylase, whereas glutamate oxaloacetate transaminase and creatine phosphokinase activities were found to have decreased. The serum proteins and free aminoacid content increased while cholesterol content decreased. Hepatic glutamate oxalo acetate transaminase, lactate dehydrogenase and isocitrate dehydrogenase activities were increased (Shakoori *et al.*, 1988).

In a study of *in vitro* covalent binding of pyrethroids namely cismethrin, cypermethrin and deltamethrin to rat liver homogenate and microsomes, it was found that inhibition of esterase and mitochondrial respiration of liver homogenate slightly altered the covalent binding level whereas inhibition of cytochrome P 450 and mixed function oxidases of microsomes reduced covalent binding (Catinot *et al.*, 1989).

The study of acute toxicity of permethrin and cypermethrin in neonatal and adult rats suggested that the higher level of sensitivity of neonate rat to pyrethroids is probably due to the incomplete development of enzymes which catalyze the metabolism of pyrethroids in liver of young animals (Cantalamessa, 1993).

A significant reduction in the levels of total serum proteins, serum gammaglobulin and a higher A: G ratio in chickens administered cypermethrin at a rate 100 ppm in feed for 8 weeks was reported by Khurana *et al.*, (1996).

Rats with multiple oral administration of cypermethrin showed a significant decrease in the blood cholinesterase activity after one week of treatment and the inhibition of cholinesterase disappeared during third week in which treatment had stopped. These findings indicate that alterations in cholinesterase levels could be used as biomarkers of pyrethroid toxicity in rats (Tong and Tian, 1996).

Garg *et al.*, (1997) observed a significant increase in the aspartate aminotransferase activities as well as concentrations of glucose and blood urea nitrogen in rats treated with fluvalinate at the rates of 35 and 70 mg/kg /day orally for 21 days.

A significant increase in the serum levels of sodium, glucose, cholesterol, alanine aminotransferase, aspartate aminotransferase and urea and a decrease in serum levels of potassium, chloride, albumin and globulin was observed in calves in chronic toxicity of cypermethrin (Patel *et al.*, 1997).

Cypermethrin, an important hepatotoxic pesticide, capable of inducing expression of apo A-1 and B genes at m RNA level but simultaneously inhibiting apo- E synthesis without causing hyperlipidemia in rats was reported by Aldana *et al.*, (1998).

Neonatal treatment with cypermethrin impaired the expression of renal dopamine D_1 and D_2 like receptors suggesting that renal dopaminergic system is a target of the toxic action of cypermethrin in rats (Cantalamessa *et al.*, 1998).

Kale *et al.*, (1999) reported that an increase in the erythrocyte lipid peroxidation is related to the inhibition of erythrocyte acetylcholine esterase activity and so acetylcholine esterase could be used as a marker enzyme in pyrethroid toxicity.

Daily dermal application of cypermethrin (0.1%) for ten consecutive days in buffalo calves produced significant inhibition of plasma cholinesterase activity, decrease in the activities of plasma aspartate aminotransferase and alanine aminotransferase, but an elevation in the activities of alkaline phosphatase and acid phosphatase (Kaur and Sandhu, 2000)

Cypermethrin was found to be cytotoxic to rat hepatocytes *in vitro* at concentrations of 200ng/ml or greater and the toxicity was measured by a decrease in the cell viability and leakage of ALT and AST enzymes into the culture medium. Cytocidal hepatotoxicity of cypermethrin in primary hepatocyte culture depends on its parent compound and pretreatment with Phenobarbital could be of therapeutic value (El- Tawil and Abdel-Rahman, 2001).

Cypermethrin exposure in rats resulted in free radical mediated damage, as indicated by elevations of cerebral and hepatic lipid peroxidation and this could be prevented by allopurinol and vitamin E (Giray *et al.*, 2001).

Patel *et al.*, (2001) observed maximum aspartate aminotransferase and alanine aminotransferase activities during 72 hours post intoxication in crossbred calves administered cypermethrin at a dose rate of 450 mg/Kg body weight orally.

The estrogenecity of organophosphorus and pyrethroid pesticides were tested and pyrethroid pesticides were found to have estrogenic activity when measured by E- screen assay, ER competitive binding assay and *p*S2 expression assay (Chen *et al.*, 2002).

Cypermethrin treatment at a low dose rate in rats for 60 days induced an increase in lipid peroxidation due to decrease in the activity of glutathione peroxidase

but decrease in membrane fluidity due to preferential localization of cypermethrin in the hydrophobic core of membrane (Gabbianelli *et al.*, 2002).

Anwar (2003) found that when different dose levels of cypermethrin were administered as a single dose an increase in the activity of serum amylase was noted on day zero but a decreased activity of serum alkaline phosphatase was observed on day seven of incubation in chickens.

Jayasree *et al.*, (2003) pointed out that deltamethrin induced oxidative damage in the biological system could be treated by supplementing vitamin E in broiler chicks.

In a study on the neurotoxic effect of dermally applied chlorpyrifos and cypermethrin in rats, Latuszynska *et al.*, (2003) reported an initial reduction in the activities of serum and brain cholinesterase and subsequent return to normal at two and three weeks post exposure.

A single oral dose toxicity of cypermethrin in rats increased malondialdehyde level and decreased activities of catalase, superoxide dismutase and glycogen level in liver but increased serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase activities (Manna *et al.*, 2004a).

Increased activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase along with elevated blood glucose level were observed in a study of repeated dose toxicity of cypermethrin in rats by Manna et al., (2004b).

Manna *et al.*, (2005b) observed increased values of serum aminotransferase, alkaline phosphatase, lactate dehydrogenase and blood glucose level in a 30 day trial of sub acute toxicity of deltamethrin in DMSO in rats.

While no statistical difference in brain and blood cholinesterase activities could be observed at a low dose level, increased brain acetylcholine esterase activities were observed in rats treated with a high dose rate of cypermethrin by Sayim *et al.*, (2005).

Manna *et al.*, (2006a) reported increased serum aminotransaminase, alkaline phosphatase (ALP), lactate dehydrogenase activities and blood glucose level in sub chronic toxicity study of alpha cypermethrin in rats.

In rainbow trout, cypermethrin induced significant elevations of plasma ammonia, aspartate aminotransferase, lactate dehydrogenase, creatine kinase and lactate, but significantly lowered values of alkaline phosphatase (Velisek *et al.*, 2006).

Chronic toxicity of cypermethrin in rats produced no significant change in liver protein values whereas liver cholinesterase activities were increased in all treatment groups (Yavasoglu *et al.*, 2006).

In a study of low dose toxicity of cypermethrin in male rats, Muthuvivekanandavel *et al.*, (2008) found increased malondialdehyde content in the brain, heart, liver, kidney and testis, increased alanine aminotransferase activities in the liver, heart and serum and increased ALP activities in the heart, kidney, testis and serum but low ALP activities in the brain and liver. While GGT activities declined in the brain, liver and serum, they were elevated in the heart, kidney and samples of testis.

2.4.3 Haematological Alterations

Reduced values of hemoglobin, red blood cell count and packed cell volume was reported by Hassan *et al.*, (1988) in rats treated with dimethoate and decamethrin orally for five months.

In a study of feeding of cypermethrin for six months in rats, the haemoglobin and leukocyte count remained unaltered while erythrocyte count and packed cell volume (PCV) decreased significantly (Shakoori *et al.*, 1988).

A significant decrease in haemoglobin values, total erythrocyte count(TEC),total leukocyte count(TLC) and PCV were reported in crossbred male buffalo calves that received cypermethrin at a dose rate of 450mg/Kg body weight orally (Patel *et al.*, 1997).

In a study of combined toxicity of cypermethrin with heavy metals namely lead and cadmium in Wistar rats, cypermethrin was found to diminish the elevation of haematocrit and mean corpuscular volume (MCV) values induced by lead and cadmium. Cypermethrin intoxication alone resulted in a significant decrease in haematocrit and MCV values (Institoris *et al.*, 1999a).

In a study of sub acute toxicity of cypermethrin and permethrin exposure in rats, Institoris *et al.*, (1999b) reported dose dependent reduction in values of haematocrit, MCV and white blood cell (WBC) counts at higher dosage by cypermethrin treatment and a decrease in MCV but increase in bone marrow cellularity in high dose group by permethrin treatment.

Reductions in the values of erythrocyte count, haemoglobin, lymphocyte count and PCV but an increase in neutrophils was observed three days after intoxication with cypermethrin in crossbred calves by Patel *et al.*, (1999).

Luty *et al.*, (2000) observed activation of leukocytic system in the form of increased number of monocytes and lymphocytes in Swiss mice when alpha cypermethrin was given orally.

An inhibition of hemopoietic system in mice with cypermethrin at a lower dose while a mobilization of hemopoietic system at a higher dose rate was reported by Haratym (2002).

Manna *et al.*, (2004b) observed a decrease in erythrocyte count, PCV and haemoglobin level in a study on repeated dose toxicity of alpha cypermethrin in rats.

Decreased values of PCV and haemoglobin were found by Manna *et al.*, (2005b) in a sub acute toxicity trial of deltamethrin in rats.

Sayim *et al.*, (2005) reported that cypermethrin treatment led to significant dose dependent decreases in RBC count, haematocrit, thrombocyte count and mean corpuscular haemoglobin values in rats.

A significant decrease in the count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes was reported in acute cypermethrin toxicity in rainbow trout by Velisek *et al.*, 2006.

2.4.4 Immunological Effects

Immunotoxicity studies of cypermethrin in rats immunized by ovalbumin and treated at the same time with 1/10 LD 50 of cypermethrin dose for 6 and 12 weeks revealed suppression of anti sheep erythrocyte titre , decreased antibody titre of blood sera and decreased the autologus rosette formation of the spleen lymphocytes (Desi *et al.*, 1986).

The ability of cypermethrin to reduce both cell mediated immunity and antibody production by lymphocytes in mice and goats was found by Tamang *et al.*, (1988).

Varshneya *et al.*, (1992) observed no adverse effects in male albino rats at low doses (5-40mg/Kg) but noticed leucopenia, significant increase in adrenal weights but decrease in spleen weights in rats receiving highest dose (40-90 mg/Kg).

Immunotoxic studies of cypermethrin revealed that cypermethrin has no effect on sheep RBC plaque forming cell count in rats, but a high dose treatment of cypermethrin produced only a slight increase in natural killer cell (NK cell) activity (Madsen *et al.*, 1996).

A significant suppression in cell mediated immunity (CMI) and humoral response in chronic cypermethrin toxicity in calves was reported by Patel *et al.*, (1997)

A significant increase in the number of peripheral blood NK cells and antibody dependent (ADCC) cytotoxic activity paralleled with a similar increase in the percentage of NK-RPI^{+} cells and decreased activity of spleen in pups when cypermethrin was given to pregnant rats (Santoni *et al.*, 1997).

Luty *et al.*, (1998) reported a significant increase in the bactericidal and phagocytic activity of neutrophils by cypermethrin intoxication through dermal route in rats.

Cypermethrin administration in rats during prenatal period affecting multiple steps in thymocyte differentiation pathways resulting in an altered cell subset

distribution and an impairment of thymocyte function was reported by Santoni *et al.*, (1998)

A decrease in the delayed type hypersensitivity reaction was observed in cypermethrin treated group whereas no effect was observed after permethrin treatment in a study designed to compare the effects of sub acute cypermethrin and permethrin exposure (Institoris *et al.*, 1999b).

Latuszynska *et al.*, (1999) observed slight elevation in the bactericidal activity of neutrophils in female Wistar rats after application of the pesticide Nurelle D550 EC for 28 days.

Cypermethrin intoxication resulted in a statistically significant increase in the phagocytic activity, but decreased bactericidal activity of neutrophils and significantly low levels of IL-12 *p*70 in Swiss mice (Luty *et al.*, 2000).

Cypermethrin induced depression in cell mediated immunity due to depletion of lymphoid cells in lymph node and spleen of crossbred calves was observed by Patel *et al.*, (2000b).

In a study of the combined toxicity of cypermethrin and arsenic in rats, it was found that the plaque formed by 10^6 spleen cells was diminished and it prolonged the time course of delayed type hypersensitivity reaction (Institoris *et al.*, 2002).

2.4.5 Genotoxicity

The genotoxicity study of cypermethrin revealed that bone marrow chromosome aberrations were not dose, time or route dependent. In the micronucleus test, the occurrence of polychromatic erythrocytes with micronucleus increased significantly with dose and there was significant difference with the control. Only marginal

differences in the incidence of sperm abnormalities were noted with different doses of cypermethrin tested in mice (Bhunya and Pati, 1988).

In a study Flodstrom *et al.*, (1988) identified fenvalerate and *p*-chlorophenyl isovaleric acid as inhibitors of intercellular communication at noncytotoxic concentrations besides suggesting fenvalerate to be a potential tumour promoter.

A more prominent sister chromatid exchange induction by cypermethrin than by deltamethrin in a trial of *in vivo* induction of sister chromatid exchange in mouse bone marrow following oral exposure to commercial formulations of alpha-cyanopyrethroids was reported by Chauhan *et al.*, (1997).

Cypermethrin caused a significant elevation in the number of metaphasic cells containing less than 42 chromosomes, but produced no effect on the number of structural aberrations while permethrin produced both the effects in a study in rats (Institoris *et al.*, 1999b).

In a study of simultaneous action of cypermethrin, cadmium and lead on the bone marrow chromosomes of rats, cypermethrin and cadmium alone caused no significant increase in the chromosomal aberrations but lead produced an elevation in the numerical aberrations. Combination of cypermethrin and cadmium produced no chromosomal effects whereas cypermethrin- lead combinations induced a significant increase in the structural chromosomal aberrations (Nehez *et al.*, 2000).

Shukla *et al.*, (2002) observed that cypermethrin possess both tumour initiating and tumour promoting potential in a long term in vivo carcinogenicity assay on mouse skin.

A study of the *in vivo* genotoxicity of synthetic pyrethroid pesticide cypermethrin in rat liver cells revealed that cypermethrin induced a clear significant positive dose dependent DNA damage in rat liver cells exposed to cypermethrin as compared with the control (El-Khatib *et al.*, 2006).

Patel *et al.*, (2006) reported a statistically significant dose dependent increase in the DNA damage in brain, kidney, liver and spleen. Brain showed maximum DNA damage followed by spleen, kidney, bone marrow, liver and finally lymphocytes.

Gross Pathology

Institoris *et al.*, (1999a) reported a decrease in the body weights of Wistar rats intoxicated with Cadmium alone as well as a combination of Cadmium and cypermethrin. The cypermethrin cadmium combination significantly increased relative liver weights.

Gross changes of congestion and haemorrhages in the auriculoventricular groove of heart, lungs, mucosal surface of intestine, cortex of kidney and brain of cypermethrin intoxicated calves was found by Patel *et al.*, (2000b).

In a study of the combined effect of cypermethrin, arsenic and mercury, significant changes in the weights of liver, kidney, adrenal and popliteal lymph node were reported by Institoris *et al.*, 2002.

In a study of acute toxicity of beta cypermethrin, Polat *et al.*, (2002) reported loss of equilibrium, rapid gill movement, erratic swimming, prolonged and motionless lying down on aquarium bottom, colour change to yellow in abdominal area, turning around its axis, enlargement of eyes and keeping gills opened for prolonged period in guppies .

Teratological abnormalities in chick embryo by cypermethrin intoxication included reduction in crown rump length, impaired development of eyes, exocardiogenesis, impaired beak development, micromelia, lack of differentiation of brain, reduction in size of brain and poor development of wing buds (Anwar, 2003).

Manna *et al.*, (2004b) observed bloat with severe haemorrhages in the stomach, haemorrhages in the intestine, pulmonary oedema, pulmonary emphysema and haemorrhages in the lungs and congestion, haemorrhages and disruption of sinusoids in the liver during a study on repeated dose toxicity of alpha cypermethrin in rats.

A significant decrease in relative brain weights of rats treated with cypermethrin at rates 60, 150 and 300 mg/kg orally for 28 consecutive days was reported by Sayim *et al.*, (2005) and a similar significant decrease in liver weight was noted by Yavasoglu *et al.*, (2006) in rats treated with 150 and 300 mg/kg cypermethrin for 28 consecutive days.

In a study of effects of oral exposure of cypermethrin on behaviour of F_1 progeny in mice, Farag *et al.*, (2007) found reduction in body weights of F_0 females at a dose rate of 10mg/kg/day and a significant reduction in maternal body weights at the end of gestation and during lactation periods whereas no effects could be seen in the progeny weight.

Teratogenecity of deltamethrin was demonstrated by the presence of various abnormalities such as prognathism, curled toe, syndactylism, crossed beak and live sticky chicks in treatment groups by Thampan (2007) in a study on pathology of deltamethrin toxicity in the chick embryo.

2.4.6 Histopathology

2.4.6.1 Liver

Hypertrophy of hepatic cells and nuclei were observed in rats that consumed cypermethrin at a dose rate of 420 mg/ kg body weight/day for six months (Shakoori *et al.*, 1988).

Cypermethrin toxicity in rats produced hepatocytes with clear vesicles of different sizes in the cytoplasm (Aldana *et al.*, 1998).

Latuszynska *et al.*, (1999) reported a few small infiltrations of lymphocytes and histiocytes between hepatocytes in Wistar rats intoxicated with Nurelle D 550 dermally.

In a study of sub acute toxicity of orally applied alpha cypermethrin in Swiss mice, Luty *et al.*,(2000) observed numerous fine lymphoid infiltrations and parenchymatous degeneration of hepatocytes.

Cypermethrin toxicity in calves induced moderate congestion of hepatic sinusoids and formation of tiny vacuoles and increased granularity in cytoplasm of hepatocytes (Patel *et al.*, 2000b).

Vascular congestion, hydropic degeneration and leukocyte infiltration during the initial stages and necrosis, perivascular and periductal fibrocellular reaction with mononuclear cell infiltration subsequently in the liver of rabbits intoxicated with cypermethrin was found by Lakkawar *et al.*, (2004).

In a single oral dose toxicity study of alpha cypermethrin in rats, Manna *et al.*, (2004a) observed congestion and haemorrhages in the liver.

Dilated sinusoids, hepatic vacuolation and Kupffer cell prominence in the liver of endosulfan treated chick embryos was reported by Kalaiselvan (2004).

Manna *et al.*, (2004b) reported congestion, haemorrhages and disruption of sinusoids of liver in a study of repeated dose toxicity of cypermethrin in rats.

Congestion and fatty changes in liver of rats intoxicated with repeated dose of deltamethrin was noted by Manna *et al.*, (2005b).

Cypermethrin intoxication in rainbow trout produced degeneration of hepatocytes, especially in peripheral zones. Affected hepatocytes showed pyknotic nuclei and many small or a single big vacuole in the cytoplasm (Velisek *et al.*, 2006).

Vacuolar degeneration with nuclear pleomorphism in the hepatocytes, dilatation of sinusoids and hepatic congestion were observed in the liver of male albino rats fed a high dose of cypermethrin orally for 28 days (Yavasoglu *et al.*, 2006).

Thampan (2007) observed sinusoidal congestion, central venous congestion, haemorrhages, vacuolation and fatty changes in the hepatocytes, necrosis, hepatocytolysis, local bile duct hyperplasia and sub capsular necrosis in the liver of chick embryos intoxicated with deltamethrin.

Low dose toxicity of cypermethrin produced vacuolation and nuclear derangement in the hepatocytes of male rats (Muthuviveganandavel *et al.*, 2008).

2.4.6.2 Brain

Postnatal exposure to deltamethrin has been observed to delay the cytogenesis

and morphogenesis of neurons of cerebellum and to induce damage to the vasculature in the form of thrombus and haemorrhage (Patro *et al.*, 1997).

Congestion and neuronal degeneration with neuronophagia in histopathological section of cerebellum in calves fed with cypermethrin at a rate of 450mg/Kg was reported by Patel *et al.*, (2000b).

Latuszynska *et al.*, (2001) observed pyknosis of brain neurocytes by combined administration of chlorpyrifos and cypermethrin in Wistar rats.

Increased density of cytoplasm in cells of cortex cerebri, stratum hippocampus, in the hilus area dentate, neurocytes of thalamus nuclei and in Purkinje cells of cerebellum of rats 3 days after dermal application of Nurelle D 550 EC was observed by Latuszynska *et al.*, (2003).

In a study of single oral dose toxicity study of alpha cypermethrin in rats, Manna *et al.*, (2004a) observed congestion and haemorrhages in the meninges and cerebellum.

Manna *et al.*, (2004b) found that repeated dose toxicity of cypermethrin in rats produced congestion and haemorrhages in the cerebellum.

Congestion of rat cerebellum after consecutive daily oral administration of deltamethrin at a rate of 15mg/kg for 30 days was reported by Manna *et al.*, (2005b).

Feeding 8 week old male Wistar rats with cypermethrin orally (60,150and 300 mg/Kg) for 28 consecutive days produced some areas of deformation due to ischemia and pyknosis of cytoplasm of neurons in brain tissue microscopically (Sayim *et al.*, 2005).

Thampan (2007) found neuronal degeneration, mild to moderate gliosis, spongiform changes in the neuropil, perivascular and perineuronal oedema, subependymal and molecular layer congestion and haemorrhage, neovascularisation of brain of chick embryos intoxicated with deltamethrin.

Vacuolation and congestion of the mesodermal layers of brain was observed in a low dose toxicity study of α cypermethrin in rats by Muthuviveganandavel *et al.*, (2008).

2.4.6.3 Stomach

Lakkawar *et al.*, (2004) reported that sub chronic toxicity of cypermethrin in rabbits produced mucosal erosion with inflammatory reaction of the gastro intestinal tract.

In a single oral toxicity study of alpha cypermethrin in rats, Manna *et al.*, (2004a) observed desquamation and necrosis of the stomach.

2.4.6.4 Intestine

Patel *et al.*, (1997) observed erosive enteritis in intestine of cypermethrin intoxicated calves.

Crossbred calves intoxicated with cypermethrin showed moderate congestion in the mucosa and sub mucosa of small intestine, degenerative changes and focal areas of desquamation of enterocytes (Patel *et al.*, 2000b).

In a study of sub chronic toxicity of cypermethrin in rats, Lakkawar *et al.*, (2004) reported lesions like mucosal eruption, congestion, leukocyte infiltration and degenerative changes in the intestine.

2.4.6.5 Lungs

In a study of cypermethrin toxicity in calves, Patel *et al.*, (1997) observed emphysema, serous exudation and thickening of interalveolar septa in lungs.

Patel *et al.*, (2000b) found mild to moderate congestion, haemorrhage, oedema and focal areas of emphysema in lungs in calves intoxicated with cypermethrin.

Haemorrhages in the lungs of rats intoxicated with a single oral dose of cypermethrin were reported by Manna *et al.*, (2004a).

In a study of repeated dose toxicity of alpha cypermethrin in rats, oedema and emphysema in lungs were observed by Manna *et a*l., (2004b).

Manna *et al.*, (2005b) reported congestion and emphysema in lungs of rats intoxicated with deltamethrin.

2.4.6.6 Heart

In a study of cypermethrin toxicity in calves, Patel *et al.*, (1997) observed moderate congestion and loss of striation of myocardial muscle fibres.

Congestion, focal areas of haemorrhage with loss of cross striation and eosinophilic appearance of cardiomyocytes were noted in the heart of calves in a cypermethrin toxicity study (Patel *et al.*, 2000b).

Muscle bands were observed to be in patches and blood vessels were found ruptured in the heart of male rats intoxicated with a low dose of cypermethrin given intradermally. (Muthuviveganandavel *et al.*, 2008)

2.4.6.7 Kidney

Latuszynska *et al.*, (1999) observed infiltrations of mononuclear cells between proximal tubules or around blood vessels of rats in a study of toxicity of dermally absorbed Nurelle D 550 EC preparations.

Kidneys of Swiss mice intoxicated with cypermethrin orally revealed few infiltrations of mononuclear cells between proximal tubules (Luty *et al.*, 2000).

In a study of cypermethrin toxicity in crossbred calves, Patel *et al.*, (2000b) observed intense congestion in the renal cortex and medulla, moderate to intense degeneration with the presence of vacuolated cytoplasm and rarefaction of the parenchyma.

Kalaiselvan (2004) reported tubular dilatation, necrosis of tubular epithelium and mesangial proliferation followed by glomerular shrinkage in chick embryo intoxicated with endosulfan.

Congestion, hydropic degeneration of tubular epithelium and leukocytic infiltration in kidneys in initial stages and hyalinization of tubular epithelium in terminal stages of cypermethrin intoxication of rabbits (Lakkawar *et al.*, 2004).

Thampan (2007) found varying degrees of glomerular and tubular cell vacuolation, tubular dilatation, tubular cell necrosis, hyalinization and calcification, generalized congestion, dilatation of Bowman's capsule and glomerular atrophy in kidneys of deltamethrin intoxicated chick embryo

Low dose toxicity of α cypermethrin produced destruction of kidney tubules in male rats (Muthuviveganandavel *et al.*, 2008).

2.4.6.8 Spleen

Depletion of lymphocytes in the Malpighian corpuscles of spleen in mice and washed out appearance of white pulp and Malpighian corpuscles in the spleen of goats in cypermethrin toxicity was found by Tamang *et al.*, (1988).

Kalaiselvan (2004) observed mild congestion in sub capsular area and vascular sclerosis in spleen of chicks in endosulfan toxicity.

The spleen of deltamethrin intoxicated chicks revealed congestion, reticuloendothelial hyperplasia, diffuse vacuolation and sub capsular depletion of cells (Thampan, 2007).

2.4.6.9 Thyroid

In a chronic cypermethrin toxicity study in goats, Gupta *et al.*, (1999) reported that thyroid gland showed decrease in staining intensity of colloid and flattening of acinar lining epithelial cells.

Histopathological examination of thyroid of cypermethrin intoxicated crossbred calves showed acini devoid of colloid along with hyperplastic and multilayered epithelial cells (Patel *et al.*, 2000a).

Materials and Methods

3. MATERIALS AND METHODS

3.1 PESTICIDE

Technical grade cypermethrin was procured from Makham Pharmachem, Bangalore.

3.2 EXPERIMENTAL ANIMALS

Adult male Sprague Dawley rats weighing approximately 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 laboratory for one week before commencement of studies.

3.3 PESTICIDE SOLUTION

Technical grade cypermethrin dissolved in 2 ml gingely oil was used for oral administration.

3.4 EXPERIMENTAL DESIGN

The animals were randomly divided into five groups of eight each and designated as groups I to V.

3.4.1 **Pesticide Administration**

Technical grade cypermethrin was dissolved in 2 ml gingely oil at specified dose rates (40 mg/kg, 80 mg/ kg and 120mg/kg body weight) and was administered orally using a gastric tube to rats of group III, group IV and group V respectively daily for 21 days.

3.4.2 **Control groups**

Group II animals were administered 2 ml gingely oil daily orally for 21 days using a gastric tube to evaluate the effect of feeding gingely oil. Group I animals were maintained without any treatment.

3.5 PARAMETERS

3.5.1 Body weight

The body weight of individual rats was recorded before the experiment (day 0) and at the end of the experiment(day 21). From this data mean body weight was noted. The animals were routinely observed for the clinical signs exhibited.

3.5.2 Blood collection

Blood samples were collected from all the animals in each group from retroorbital plexuses after anaesthetizing animals with diethyl ether. Blood was collected with appropriate anticoagulant for estimation of haematological parameters. Blood sample without anticoagulant was collected on day 0 and day 21 of the experiment for the estimation of serum biochemical parameters. EDTA was used as anticoagulant at the rate of 2 mg/ml.

3.5.3 Haematological parameters

Total leukocyte count (TLC) and packed cell volume (PCV) were estimated by the method suggested by Thrall *et al.,* (2004) on days 0,7,14,21. Total erythrocyte count (TEC) and concentration of haemoglobin (Hb) by acid haematin method were determined as described by Feldman *et al.,* (2000) on days 0, 7, 14, 21. Differential leukocyte count (DLC) was estimated on day 21 of the experiment by the method suggested by Thrall *et al.,* (2004).

3.5.4 Biochemical studies

Blood collected in fresh vials without anticoagulant was kept at room temperature for one hour. Then it was centrifuged at 2000 rpm for 20 minutes. The serum was aspirated into another vial and used for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl

transferase (GGT) on day 0 and day 21 of the experiment. Serum was collected for the estimation of total protein and albumin on day 21 of the experiment.

Estimation of serum AST and ALT was done using kits manufactured by Agappe diagnostics (Reitman and Frankel, 1957). Gamma glutamyl transferase in serum was estimated using kits manufactured by Agappe Diagnostics (Alan, 1988). Total serum protein was estimated by Biuret method (Henry *et al.*, 1957). Albumin was estimated by Doumas method (Doumas *et al.*, 1971) using kit supplied by Agappe Diagnostics.

3.6 PATHOANATOMICAL STUDIES

At the end of the experiment, animals were sacrificed. Detailed postmortem examination was conducted and gross lesions were noted. Brain, spinal cord, oesophagus, stomach, intestine, liver, kidney, lungs and heart were collected for histopathology. Brain and spinal cord were fixed in neutral buffered formalin and other tissues were fixed in 10% formalin. Sections were cut at 4 micron thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Gamble, 2002).

3.7 STATISTICAL ANALYSIS

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

Results

4. RESULTS

Results obtained from cypermethrin toxicity experiment on rats were tabulated and presented in tables and graphs/ diagrams.

4.1 MEAN BODY WEIGHT

The average body weight of animals on the first day and last day are shown in table 1. The mean body weight of animals in group I, III and IV did not differ significantly, whereas Group II showed significant difference in body weight ($p< 0.05$) and is shown in Fig. 1.

4.2 HAEMATOLOGICAL PARAMETERS

4.2.1 Packed Cell Volume (PCV)

The average packed cell volume of each group at weekly intervals is shown in table 2. A significant difference in PCV values between groups and period could be observed at 5% level (Fig.2). Mean PCV of control group was 46.5±0.42%and low dose and medium dose groups had a PCV of 41.25±0.49% and 39±1% respectively at the end of the experiment.

4.2.2 Haemoglobin Concentration

A gradual dose and time dependent reduction in haemoglobin level could be observed in the treatment groups as compared with the control groups (table 3). There was a significant variation in haemoglobin concentration at 5% level (Fig.3). The haemoglobin concentration of control group was 17±0.01g/dl at the end of the experiment whereas it was reduced to 15.02±0.16g/dl and 14.2±0.4g/dl in low dose and medium dose groups respectively.

4.2.3 Total Erythrocyte Count (TEC)

The mean erythrocyte count of Group I, II, III and IV at weekly intervals is given in table 4 and graphically in Fig 4. The data revealed a significant difference in erythrocyte count between groups and period of treatment at 5% level. The TEC of treatment groups were $7.12\pm0.17x10^6/\mu l$ and $6.87\pm0.1x10^6/\mu l$ for low dose and medium dose group respectively and for control group TEC was $8.28\pm0.05x10^6/\mu l$.

4.2.4 Total Leukocyte Count (TLC)

The leukocyte count of treatment groups at different periods varied significantly as compared with the control groups at $p<0.05$ (Fig.5). The leukocyte count at different intervals is presented in table 5. The TLC of control group (GroupI) was 8.62 ± 0.01 thousands $/mm^3$and that of low dose and medium dose groups were 7.53 ± 0.02 thousands $/mm^3$and 6.78 ± 0.03 thousands$/mm^3$ respectively.

4.2.4 Differential Leukocyte Count (DLC)

The DLC revealed neutropenia and lymphocytosis in treatment groups (Fig.6). The neutrophil and lymphocyte count is presented in table 6. The differential counts expressed in percentage vary significantly between groups at $p<0.05$. The treatment groups were found to have neutropenia ($15.38\pm0.92\%$ and $10.5\pm1.5\%$ in low dose and medium dose groups respectively) as compared with the neutrophil count of the control group ($18.63\pm1.05\%$). The lymphocyte count of control group was $80.13\pm1.14\%$ whereas that of low dose group was $82.5\pm1.12\%$ and medium dose group was $89.5\pm1.5\%$.

4.3 BIOCHEMICAL PARAMETERS

4.3.1 Serum Alanine Aminotransferase (ALT)

The values of ALT are presented in table 7 and graphically in Fig. 7. A significant increase in the level of ALT was noted in cypermethrin treated groups as

compared with the control groups at 5% level. At the end of the experiment, the ALT level of the control group was estimated to be 56.87±2.4 IU/L. The ALT level of the low dose group was elevated to 130.12±4. 29 IU/L and for the medium dose group ALT level was 209.5±13.5 IU/L.

4.3.2 Serum Aspartate Aminotransferase (AST)

Results of aspartate aminotransferase assay are presented in table 8 and graphically in Fig. 8. Results indicated that cypermethrin produced a significant increase in the aspartate aminotransferase level in the serum of animals of group III and IV at 5% level. The AST levels were 175.37±2.9 IU/L, 268.87±2.89IU/L, 271.5±12.5 IU/L for control, low dose and medium dose groups respectively.

4.3.3 Serum Gamma Glutamyl Transferase (GGT)

The gamma glutamyl transferase level of treatment groups showed no significant difference as compared with the control groups at $p<0.05$.(Table 9). The GGT level of all groups ranged from 0-2 IU/L.

4.3.4 Serum Total Protein

The average total protein content of groups I, II, III and IV is depicted in table 10 and graphically in Fig. 9. A significant reduction in the total protein content of group III and IV could be observed at 5% level. The control animals showed serum total protein content of 6.54 ±0.09 g and in low dose and medium dose groups it was reduced to 4.89±0.17 g and 4.6 ±0.01 g respectively.

4.3.5 Serum Albumin

Results of estimation of the albumin level are presented in table 11. The treatment groups (Group III and IV) showed a significant reduction in the albumin content as compared with the control groups at $p<0.05$ (Fig. 10). The serum albumin

content of low dose and medium dose groups were 3.12±0.11g and 2.75 ±0.05 g respectively whereas that of the control group was 3.46±0.08 g.

4.3.6 Serum Globulin

A gradual dose dependent reduction in the globulin level could be observed at 5% level and it is presented in table 11 and Fig. 10. The globulin level of the control group was 3.01±0.1g. The globulin content of low dose and medium dose groups were 1.76±0.19g and 1.85±0.05 g respectively.

4.3.7 A: G Ratio

No significant difference in A: G ratio between control groups and treatment groups could be observed at $p<0.05$ (Table 11). A: G ratio of all groups ranged from 1-2. It is presented in Fig. 11.

4.4 CLINICAL SIGNS

The clinical signs shown by animals in Group IV (medium dose) and Group V (high dose) were almost similar. The animals exhibited burrowing behaviour, salivation, somnolence (Fig.12), gradual development of hind limb extensor tone (Fig. 13), elevated startled response and seizures. Choreoathetosis developed and as symptoms progressed choreoathetosis became continuous and the animal went into lateral recumbency. During the terminal stages, animals showed laboured breathing and gasping. The animals in Group III (low dose group) showed salivation, somnolence, burrowing behaviour and weakness. Few animals in this group developed hind limb extensor tone in the initial days following intoxication and gradually it recovered.

4.5 MORTALITY PATTERN

A dose related pattern of mortality could be observed in the cypermethrin treated groups. The group which received the highest dose (group V) showed 100% mortality and all the animals died within 3-7 days. The medium dosage group (group IV) also showed a high percentage of mortality and only 2 animals remained alive at the end of the experiment. The mortality occurred between 6-10 days in most of the cases in group IV. No mortality was observed in the control and the low dosage groups.

4.6 PATHOANATOMICAL STUDIES

4.6.1 Gross Pathology

4.6.1.1 Group I

The animals were sacrificed on 21st day of experiment. The animals maintained without any treatment revealed no apparent gross lesions during post mortem and tissues for histopathology were collected within ten minutes.

4.6.1.2 Group II

The animals fed with gingely oil alone were sacrificed and detailed post mortem conducted. In these animals marked enlargement of the liver could be observed. All other organs examined appeared apparently normal.

4.6.1.3 Group III

Bloat and congestion of gastric mucosa could be observed in the stomach of animals fed with low dose cypermethrin for 21 days. Other lesions observed include congestion in the lungs, brain, liver and kidney. In some of the animals examined, mild catarrhal enteritis in the intestines and degenerative changes in the liver evidenced in the form of cooked appearance could be observed.

4.6.1.4 Group IV

In this group, individual mortality was recorded on days 1, 2, 5, 8, 10 and 19 of the experiment. The gross lesions in dead animals were bloat, congestion of heart, pulmonary congestion along with extensive haemorrhages (Fig. 14) in diaphragmatic lobes, slightly swollen and pale appearance of liver. An ulcer was observed on glandular portion of stomach of animal that died on day 19 of the experiment (Fig.15). Other lesions were same in this animal. The gross lesions in animals sacrificed after 21 days of cypermethrin intoxication include congestion of the lungs, brain, gastric mucosa and liver.

4.6.1.5 Group V

The mortality pattern of this group was as follows. Two animals were found dead on day 3, four animals on day 6 and two animals on day 7 of intoxication with 120 mg/kg body weight of cypermethrin. Postmortem lesions included bloat, congestion of the lungs, heart and pulmonary haemorrhage (Fig.17). Congestion and oedema was observed in brain (Fig. 16). Degenerative changes and congestion in the liver (Fig.18) and kidney could be observed.

4.6.2 Histopathology

4.6.2.1 Group I

Brain, spinal cord, oesophagus, stomach, intestine, liver, kidney, lungs and heart were collected at the end of the experiment. The microscopic lesion observed was mild congestion of the liver, lungs and heart.

4.6.2.2 Group II

Liver of vehicle treated animals revealed vacuolation of hepatocytes (Fig.19). Lungs, liver and heart revealed mild congestion.

4.6.2.3 Group III

Brain revealed moderate gliosis, perineuronal vacuolation, perivascular vacuolation and neuronal degeneration (Fig. 22). Spinal cord showed neuronal degeneration, widening of the perineuronal space and vacuolation of the white matter (Figs. 20 & 21). Early degenerative changes and subsequent necrotic changes were evident in the liver sections (Fig.24). There was Kupffer cell hyperplasia and central venous stasis (Fig. 25). Intestines revealed goblet cell hyperplasia, fusion of villi and inflammatory cell infiltration into the lamina propria and sub mucosa (Fig. 23). There was pulmonary congestion and haemorrhage in the lung (Fig. 28). In some cases, the lung showed peribronchial congestion, lymph stasis and emphysema. In kidney there was diffuse degeneration of the tubular lining cells, congestion, haemorrhage and diffuse atrophy of glomeruli (Figs. 26 & 27). Oesophagus, stomach and heart sections revealed no lesions.

4.6.2.4 Group IV

Brain revealed shrunken neurons, perineuronal vacuolation, perivascular oedema, moderate spongiosis and moderate gliosis (Fig.29). Diffuse degeneration of hepatocytes, focal necrosis with mononuclear cell infiltration (Fig. 37) and central venous congestion were observed in the liver (Fig. 36). Intestines showed fusion of villi, diffuse hyperplasia of the goblet cells with focal infiltration of inflammatory cells into the lamina propria and sub mucosa (Figs. 31& 32). In the kidney, diffuse tubular necrosis (Fig. 35), multiple haemorrhages both in the cortical and medullary tubules besides interstitium were observed (Fig. 33). Occasional atrophy of the glomeruli was observed. In some proximal convoluted tubules, there was desquamation and hyalinization of tubular epithelial cells (Fig. 34). Oedema and haemorrhages were observed in the lungs (Fig. 38). There was intramuscular haemorrhage, myolysis and congestion in the heart (Fig. 30). Stomach of one animal

revealed discontinuity of epithelium with the presence of RBC. Spinal cord and oesophagus revealed no lesions.

4.6.2.5 Group V

In brain, congestion in meninges and cerebrum, perivascular vacuolation (Fig.39), perineuronal vacuolation,spongiosis of white matter and gliosis (Fig. 41) were observed. Discontinuity and shrinkage of Purkinje cells in cerebellum was also observed (Fig. 40). In the liver, dissociation of hepatic cords (Fig. 49) dilatation of sinusoids, lymph stasis, and central venous congestion were evident (Fig.48). There was superficial erosion with presence of erythrocytes and lysed cells in the non glandular portions of stomach (Fig. 52). Focal hyperkeratinisation and focal proliferation of stratified squamous epithelium of non glandular portions was also observed (Fig. 53). Intestines revealed extensive necrosis with infiltration of inflammatory cells (Figs. 44 & 45). Oesophagus appeared to have collapsed lumen. In the kidneys, there was congestion, haemorrhage, tubular degeneration and necrosis (Figs. 46 & 47). Lungs revealed pulmonary oedema, congestion, haemorrhage (Fig. 51) and perivascular oedema (Fig. 52). Diffuse congestion, haemorrhage and focal myolysis were observed in the heart (Figs. 42 & 43). Spinal cord revealed no lesion.

Table 1. Mean weight of animals

GROUPS	Day0 (g)	Day 21 (g)
I	$199.37^{a}\pm1.75$	$226.88^{a}\pm1.31$
II	$200.62^{a}\pm2.2$	$242.5^{b}\pm1.64$
III	$201.12^{a}\pm1.62$	$221.88^{a}\pm1.62$
IV	$200.62^{a}\pm2.2$	$219.5^{a}\pm2.5$

(Means bearing same superscript does not differ significantly)

Table 2.Mean packed cell volume

GROUPS	PACKED CELL VOLUME (%)			
	Day 0	Day 7	Day 14	Day 21
I	$46.37^{a}\pm0.94$	$46.63^{a}\pm0.87$	$47.13^{a}\pm0.67$	$46.5^{a}\pm0.42$
II	$47.25^{a}\pm0.45$	$47.5^{a}\pm0.57$	$48^{a}\pm0.73$	$48.25^{a}\pm0.88$
III	$46.75^{a}\pm0.53$	$46.88^{a}\pm0.72$	$43.13^{b}\pm1.11$	$41.25^{b}\pm0.49$
IV	$47^{a}\pm1.13$	$42.71^{b}\pm1.63$	$41^{b}\pm1.53$	$39^{c}\pm1$

(Means bearing same superscript does not differ significantly)

Table 3. Mean haemoglobin concentration

GROUPS	HAEMOGLOBIN CONCENTRATION (g /dl)			
	Day 0	Day 7	Day 14	Day 21
I	$17.05^{a}\pm0.10$	$17.02^{a}\pm0.09$	$16.8^{a}\pm0.1$	$17^{a}\pm0.01$
II	$16.92^{a}\pm0.16$	$16.85^{a}\pm0.13$	$17.15^{a}\pm0.11$	$17.4^{a}\pm0.14$
III	$17.05^{a}\pm0.12$	$16.15^{a}\pm0.13$	$15.89^{b}\pm0.28$	$15.02^{b}\pm0.16$
IV	$17.02^{a}\pm0.07$	$16.11^{a}\pm0.15$	$15.21^{b}\pm0.12$	$14.2^{c}\pm0.4$

(Means bearing same superscript does not differ significantly)

Table 4. Mean total erythrocyte count

GROUPS	TOTAL ERYTHROCYTE COUNT (10^{6}/µl)			
	Day 0	Day 7	Day 14	Day 21
I	$8.32^{a}\pm0.13$	$8.3^{a}\pm0.08$	$8.43^{a}\pm0.07$	$8.28^{a}\pm0.05$
II	$8.63^{a}\pm0.11$	$8.6^{a}\pm0.13$	$8.63^{a}\pm0.06$	$8.67^{a}\pm0.09$
III	$8.4^{a}\pm0.16$	$7.9^{b}\pm0.13$	$7.63^{b}\pm0.21$	$7.12^{b}\pm0.17$
IV	$8.68^{a}\pm0.06$	$7.71^{b}\pm0.14$	$7.17^{b}\pm0.07$	$6.87^{c}\pm0.1$

(Means bearing same superscript does not differ significantly)

Table 5. Mean total leukocyte count

GROUPS	TOTAL LEUKOCYTE COUNT (thousands per mm^3)			
	Day 0	Day 7	Day 14	Day 21
I	8.63^{a}±0.13	8.66^{a}±0.01	8.7^{a}±0.01	8.62^{a}±0.01
II	8.69^{a}±0.08	8.63^{a}±0.07	8.69^{a}±0.05	8.74^{a}±0.11
III	8.61^{a}±0.07	8.34^{a}±0.01	7.93^{b}±0.18	7.53^{b}±0.02
IV	8.61^{a}±0.08	7.83^{b}±0.01	7.23^{b}±0.12	6.78^{c}±0.03

(Means bearing same superscript does not differ significantly)

Table 6. Mean Differential Leukocyte count on day 21

GROUPS	NEUTROPHIL (%)	LYMPHOCYTE (%)
I	18.63^{a}±1.05	80.13^{a}±1.14
II	20.13^{a}±1.11	77.12^{a}±1.42
III	15.38^{b}±0.92	82.5^{b}±1.12
IV	10.5^{c}±1.5	89.5^{c}±1.5

(Means bearing same superscript does not differ significantly)

Table 7. Mean values of alanine aminotransferase

GROUPS	ALANINE AMINOTRANSFERASE(IU/L)	
	Day 0	Day 21
I	$57.63^{a}\pm1.16$	$56.87^{a}\pm2.4$
II	$58.38^{a}\pm2.03$	$58.75^{a}\pm1.47$
III	$63.63^{a}\pm2.01$	$130.12^{b}\pm4.29$
IV	$54.02^{a}\pm2.8$	$209.5^{c}\pm13.5$

(Means bearing same superscript does not differ significantly)

Table 8. Mean values of aspartate aminotransferase

GROUPS	ASPARTATE AMINOTRANSFERASE(IU/L)	
	Day 0	Day 21
I	$181.75^{a}\pm2.22$	$175.37^{a}\pm2.9$
II	$182.12^{a}\pm4.8$	$175.25^{a}\pm2.81$
III	$186.5^{a}\pm2.26$	$268.87^{b}\pm2.89$
IV	$184.75^{a}\pm6.3$	$271.5^{b}\pm12.5$

(Means bearing same superscript does not differ significantly)

Table 9. Mean values of gamma glutamyltransferase

GROUPS	GAMMA GLUTAMYLTRANSFERASE(IU/L)	
	Day 0	Day 21
I	0.75±0.25	0.25±0.16
II	0.5±0.19	0.25±O.46
III	0.25±0.16	1.63±0.38
IV	0.5±0.25	2±0.51

Table 10. Mean total protein on day 21

GROUP	TOTAL PROTEIN (g)
I	6.54^{a}±0.09
II	6.5^{a}±0.09
III	4.89^{b}±0.17
IV	4.6^{b}±0.01

(Means bearing same superscript does not differ significantly)

Table 11. Mean serum proteins on day 21

GROUP	ALBUMIN(g)	GLOBULIN(g)	A:G
I	$3.46^{a}\pm0.08$	$3.01^{a}\pm0.10$	$1.16^{a}\pm0.06$
II	$3.62^{a}\pm0.05$	$2.88^{a}\pm0.07$	$1.2^{a}\pm0.04$
III	$3.12^{b}\pm0.11$	$1.76^{b}\pm0.19$	$2.01^{a}\pm0.36$
IV	$2.75^{c}\pm0.05$	$1.85^{b}\pm0.05$	$1.45^{a}\pm0.05$

(Means bearing same superscript does not differ significantly)

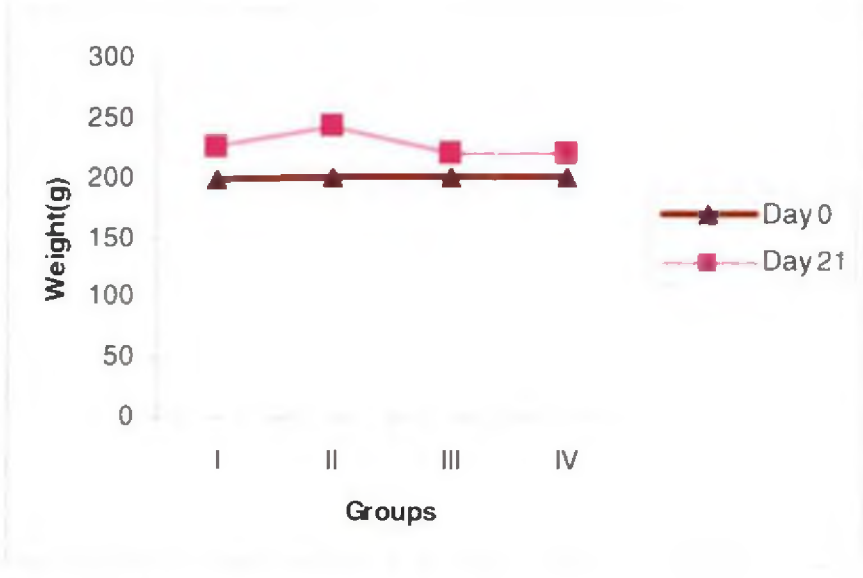


Fig.1 **Mean weight of animals**

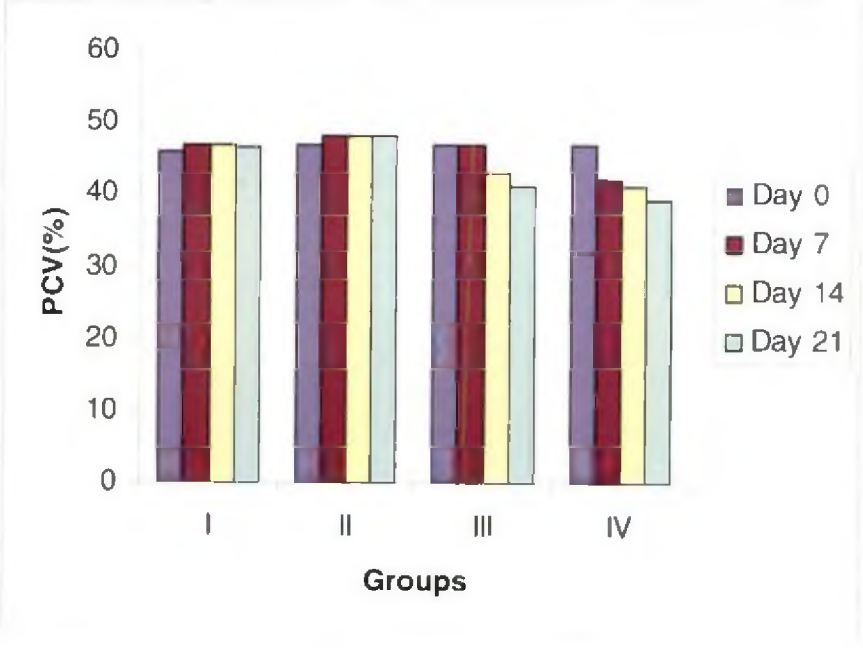


Fig.2 **Packed cell volume**

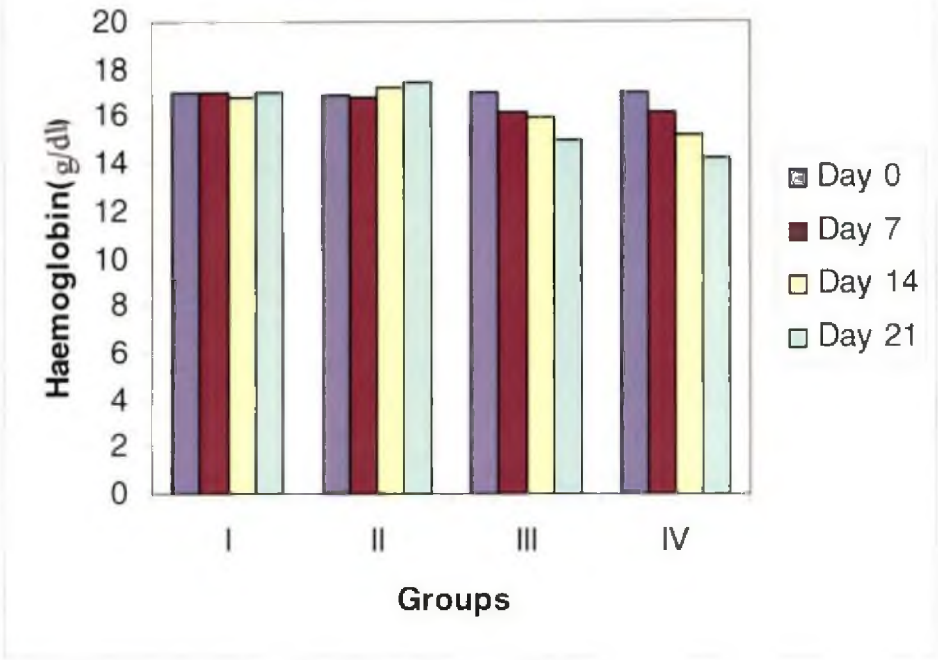


Fig.3 **Haemoglobin concentration**

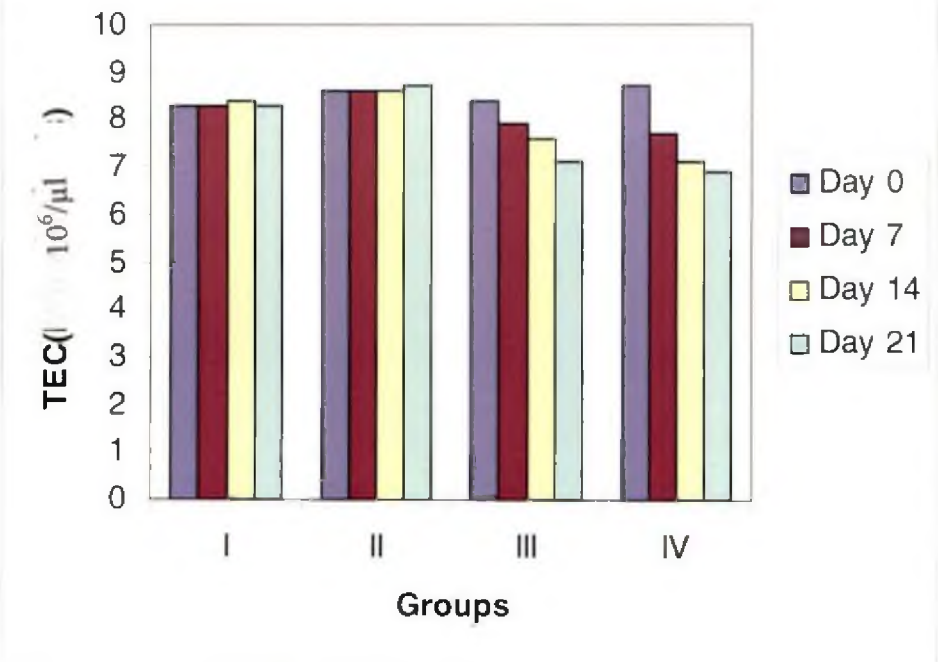


Fig.4 **Total erythrocyte count**

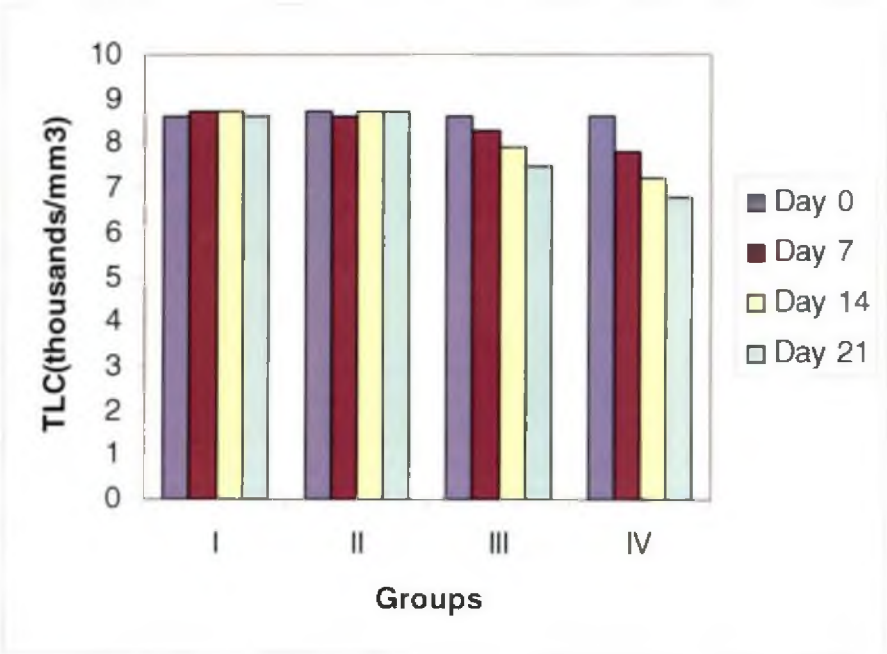


Fig.5 **Total leukocyte count**

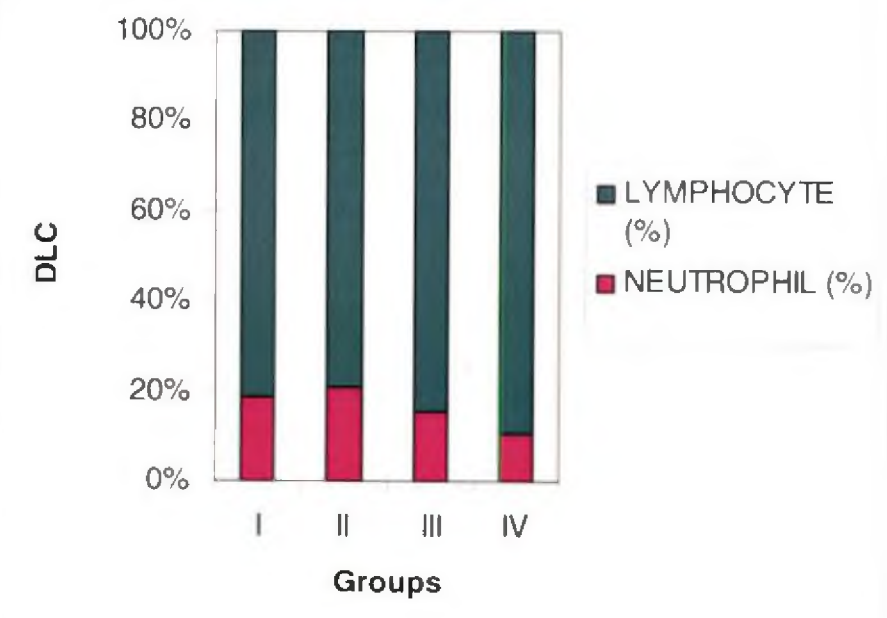


Fig.6 **Differential leukocyte count**

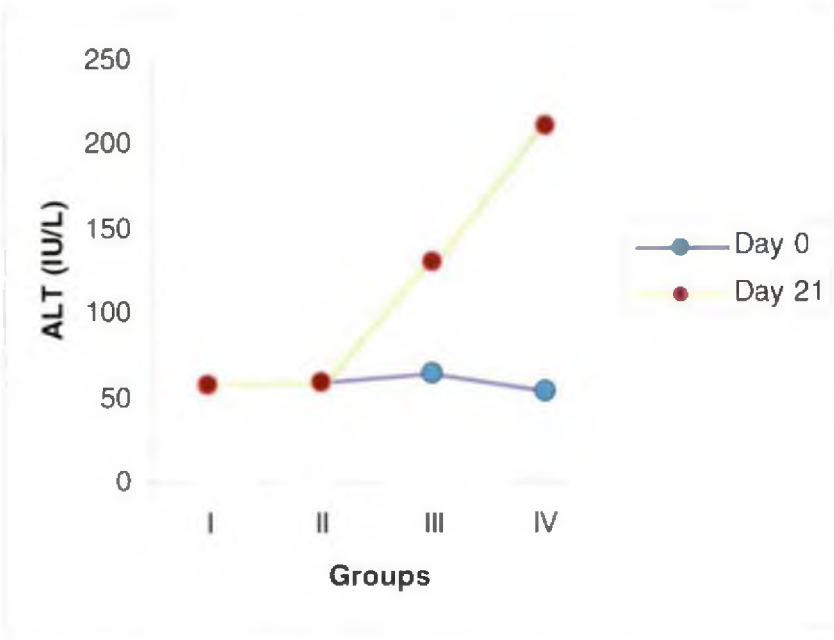


Fig.7 **Alanine aminotransferase estimation**

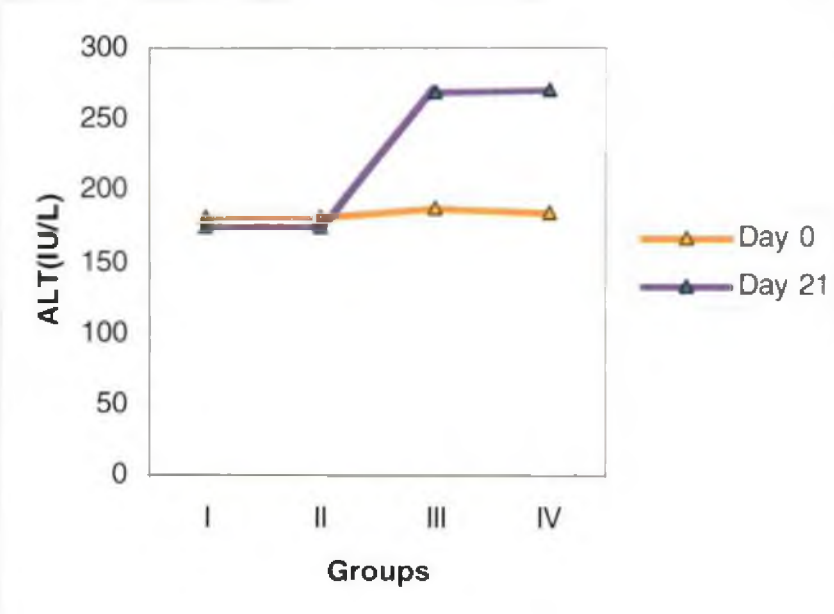


Fig.8 **Aspartate aminotransferase estimation**

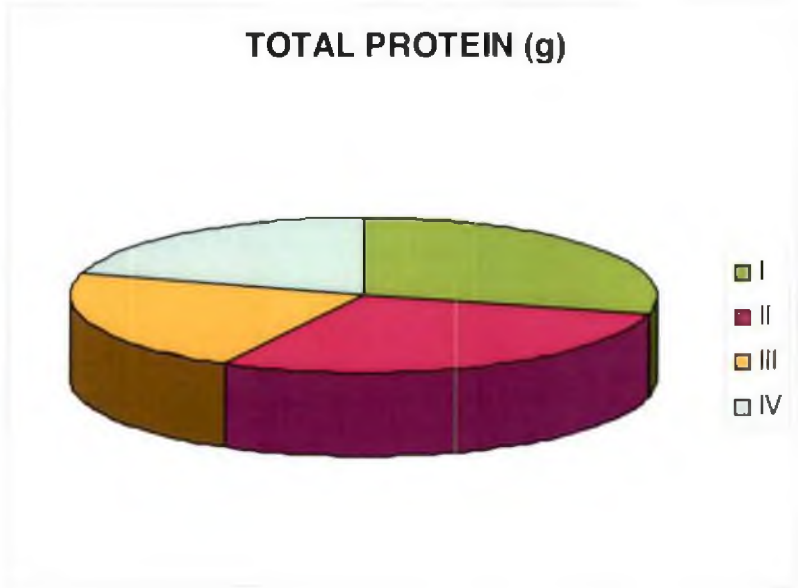


Fig.9 **Total serum protein**

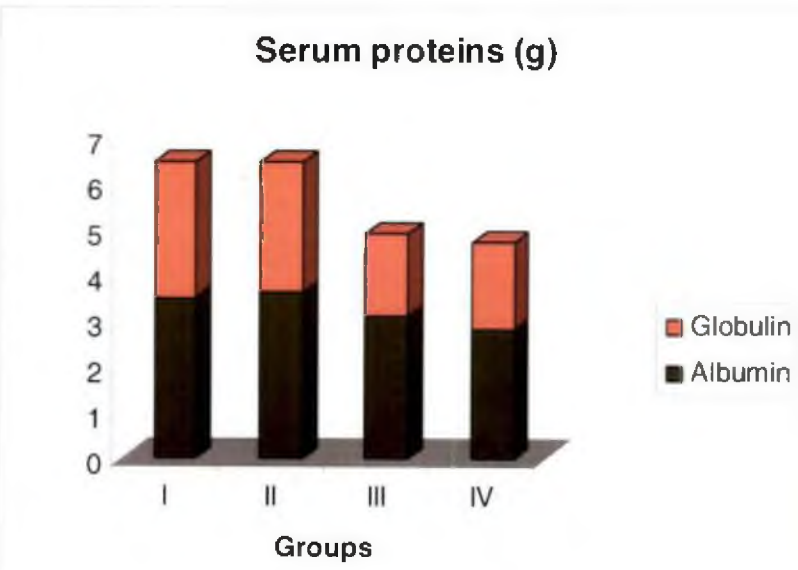


Fig.10 **Serum proteins**

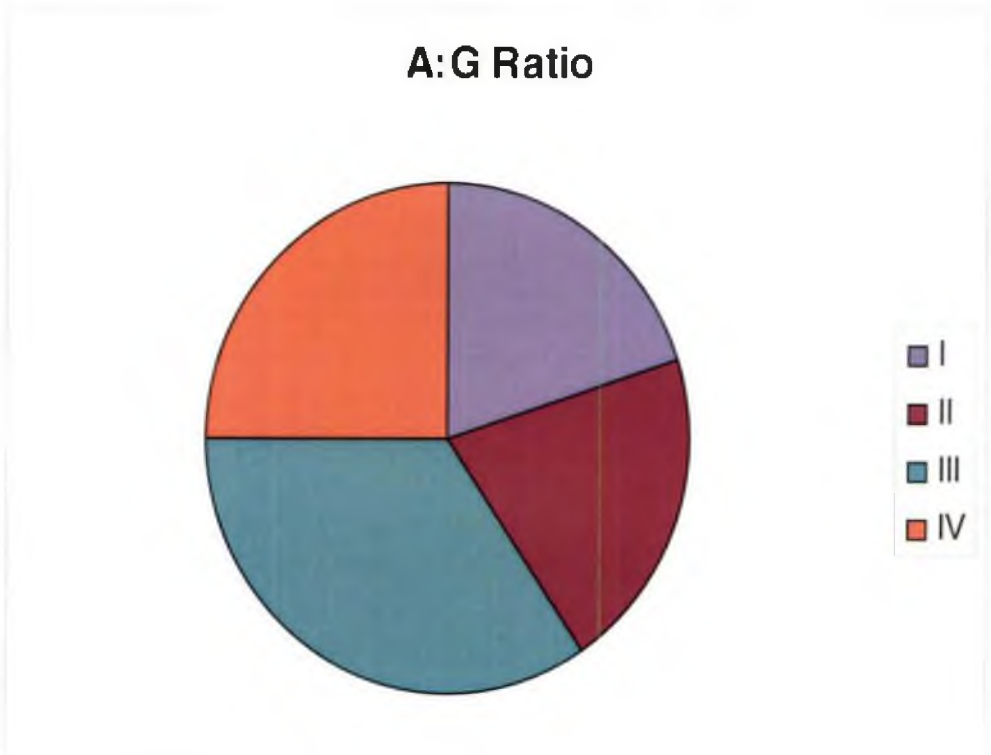


Fig.11 **A: G Ratio**

Fig.12

Fig.13

Fig.12 Somnolence, 80mg/kg body weight

Fig.13 - Extensor tone reflex, 80 mg/kg body weight

Fig.14

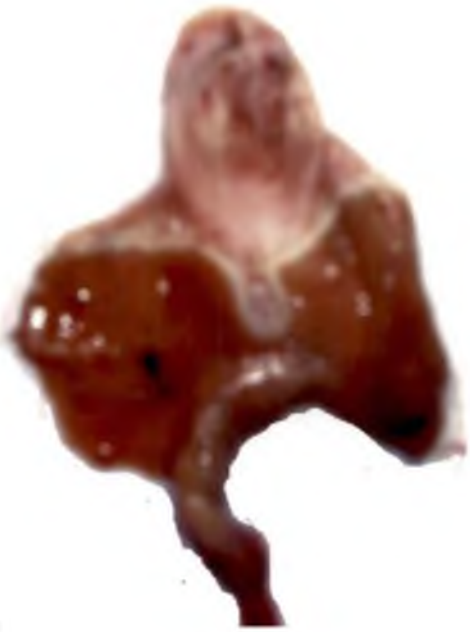


Fig.15

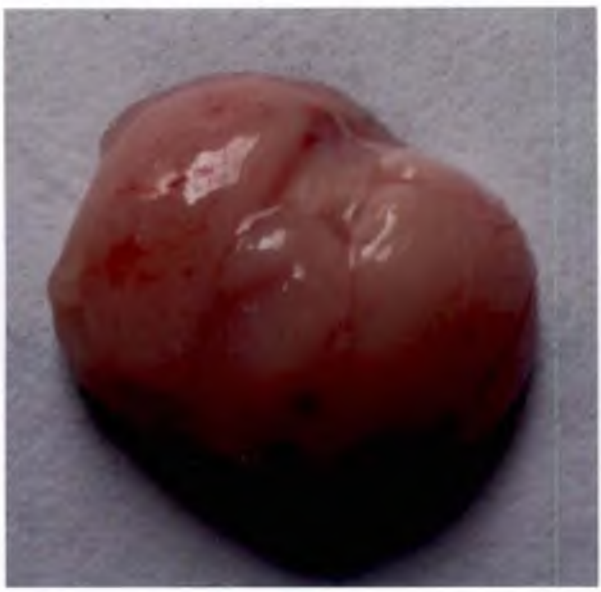


Fig.16

Fig.17

Fig.14- Lungs-Congestion and sub pleural haemorrhage, 80 mg/kg body weight

Fig.15- Stomach-Ulcer in glandular portion, 80 mg/kg body weight

Fig.16- Brain-Congestion and oedema, 120 mg/kg body weight

Fig.17- Lungs-Extensive subpleural haemorrhage, 120 mg/kg body weight

Fig.18

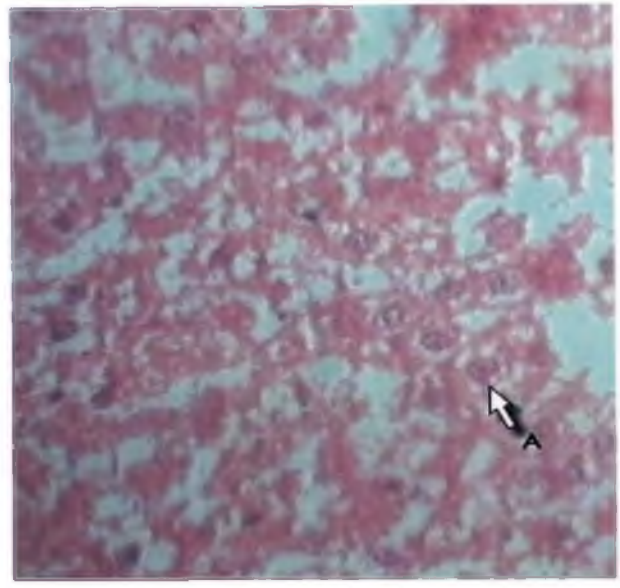


Fig.19

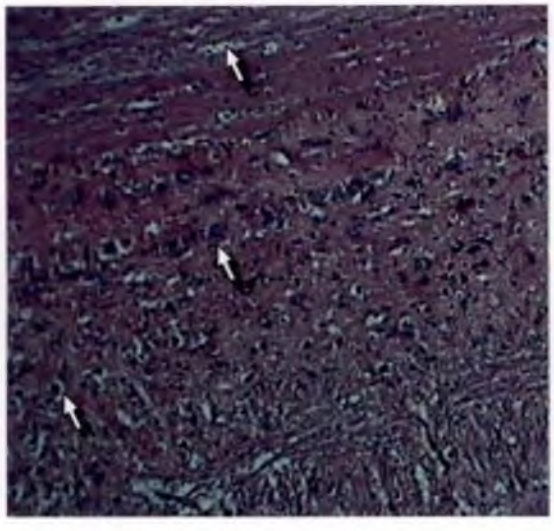


Fig.20

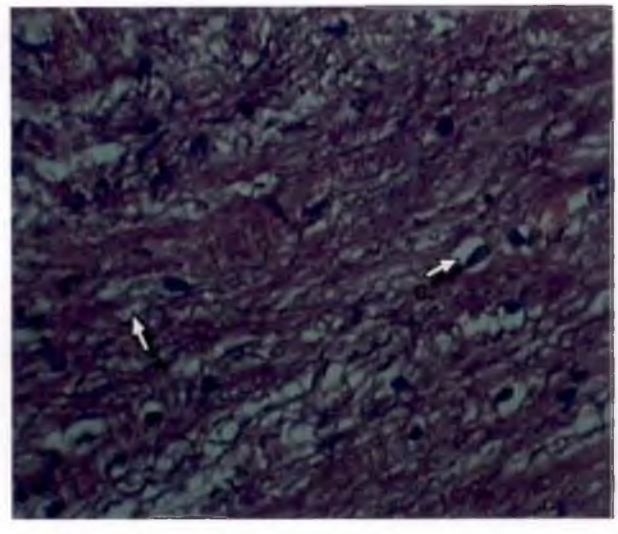


Fig.21

Fig.18- Liver – Patchy areas of degeneration, 120 mg/kg body weight

Fig.19- Liver - Vacuolation on hepatocyte cytoplasm (A), group II - H & E,x 400

Fig.20 - Spinal cord - Perineuronal vacuolation (A), degeneration of large neurons (B), white matter vacuolation (C), 40 mg/kg body weight - H & E x 100

Fig.21 - Spinal cord - Spongiosis of white matter (A), perineuronal vacuolation (B), 40 mg/kg body weight - H & E x 400

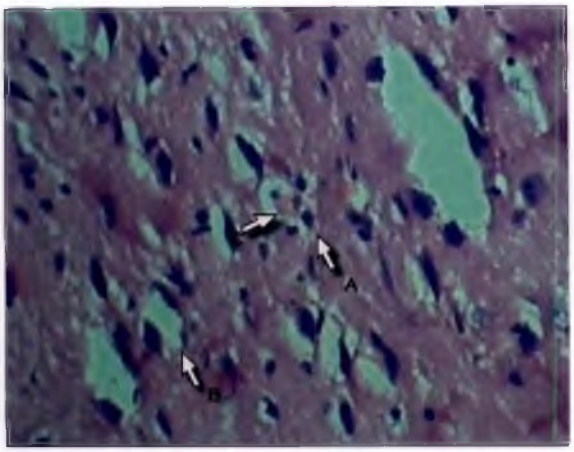
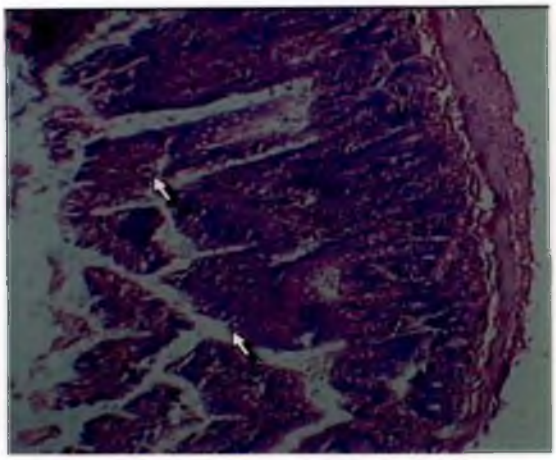


Fig.22



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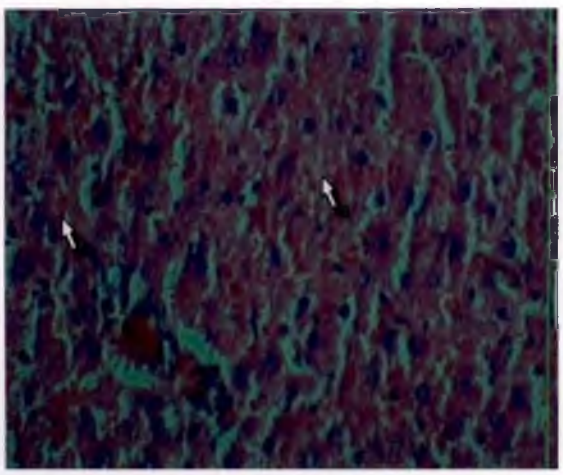

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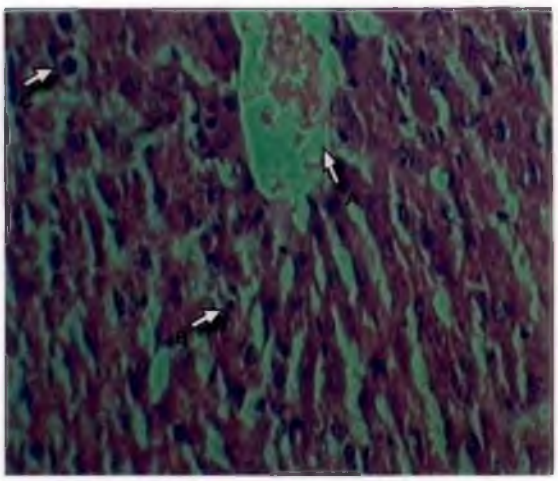


Fig.25

Fig.22 - Brain - Gliosis (A), perineuronal vacuolation (B), neuronal degeneration (C), 40 mg/kg body weight - H & E x 400

Fig.23 - Intestine - Inflammatory cell inflitration (A), goblet cell hyperplasia (C), 40 mg/kg body weight - H & E x 100

Fig.24 - Liver - Sinusoidal congestion (A), necrotic changes in hepatocytes (B), 40 mg/kg body weight - H & E x 400

Fig.25 - Liver - Congestion (A), Kupffer cell hyperplasia (B), vacuolation in hepatocyte cytoplasm (C), 40 mg/kg body weight - H & E x 400

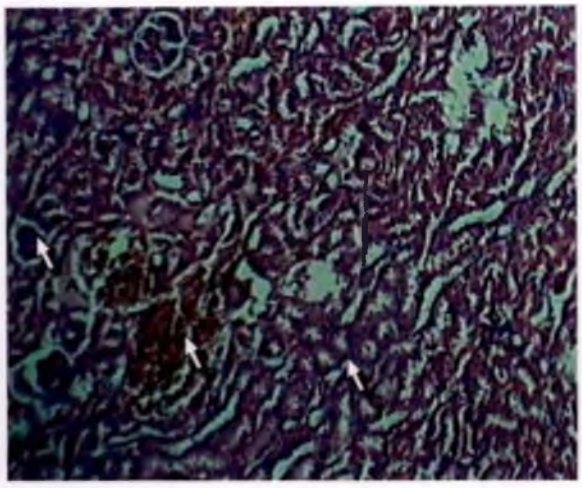


Fig. 26

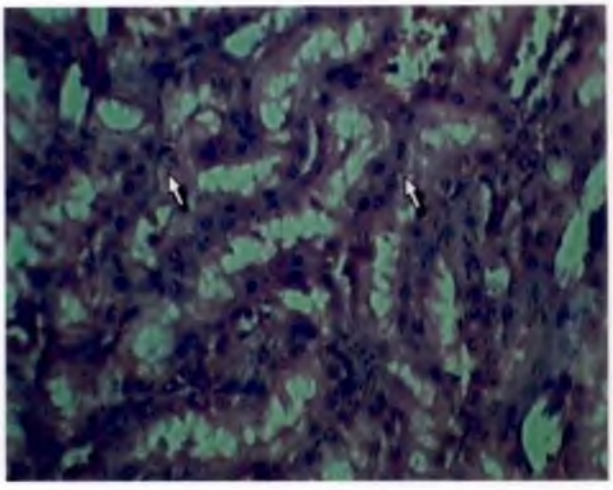


Fig.27

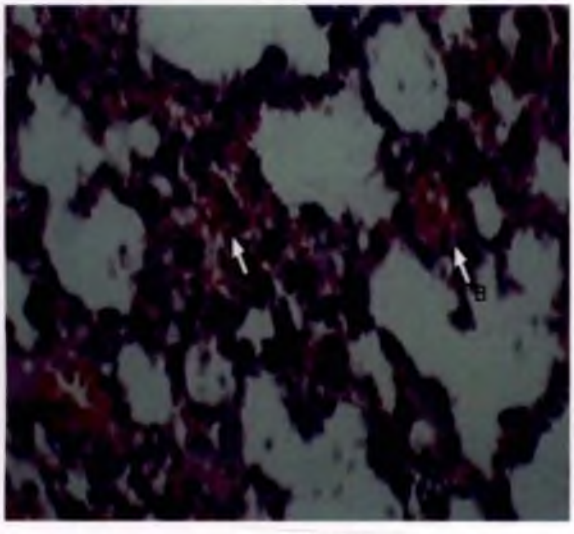


Fig.28

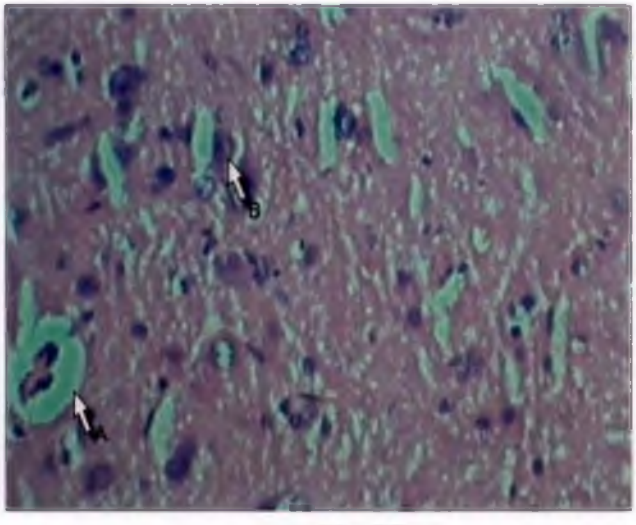


Fig.29

Fig. 26- Kidney - Atrophy of glomeruli (A), haemorrhage (B), degeneration of tubular epithelial cells (C), 40 mg/kg body weight - H & E x 100

Fig.27- Kidney - Vacuolation on tubular epithelial cells (Arrow), 40mg/kg body weight - H & E x 400

Fig.28- Lungs - Pulmonary haemorrhage (A), congestion (B), 40 mg/kg body weight - H & E x 400

Fig.29 - Brain - Perivascular vacuolation (A), perineuronal vacuolation (B), 80 mg/kg body weight - H & E x 400

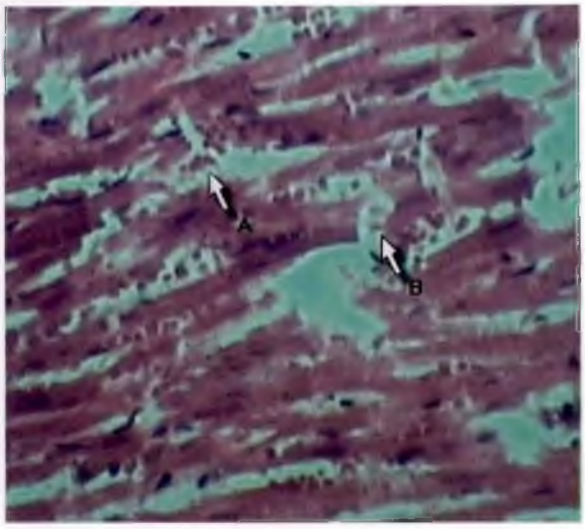


Fig.30

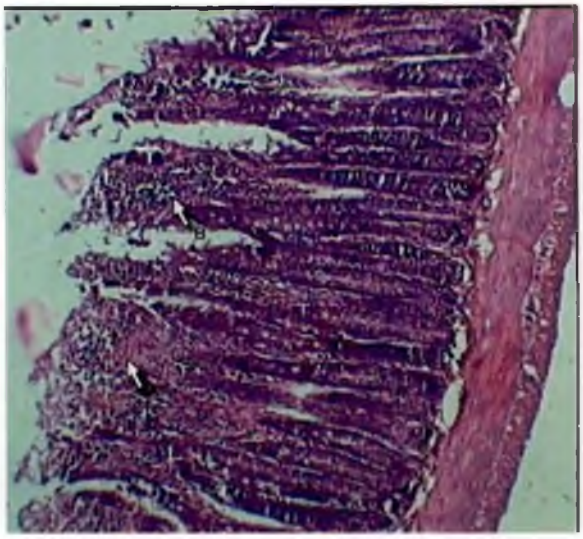


Fig. 31

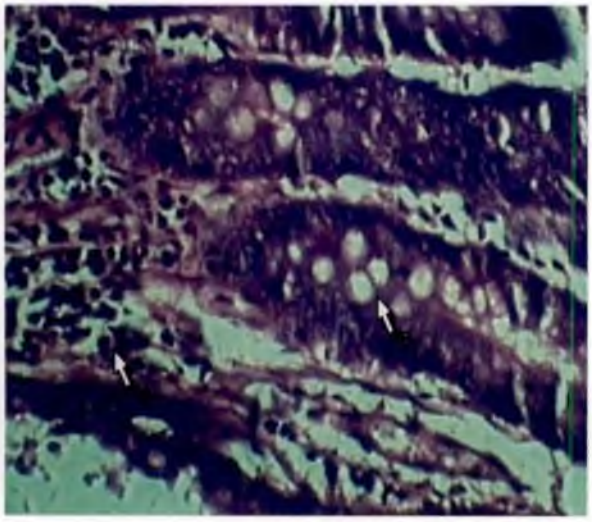


Fig. 32

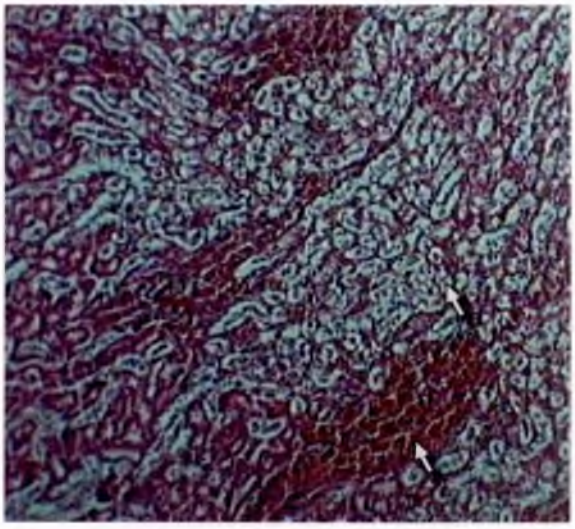


Fig.33

Fig.30 - Heart - Inter muscular haemorrhage (A), myolysis (B), 80 mg/kg body weight - H & E x 400

Fig. 31 - Intestine - Fusion of villi (A), inflammatory cell in filtration (B), 80 mg/kg body weight - H & E x 100

Fig. 32 - Intestine - Inflammatory cell in filtration (A), goblet cell hyperplasia (B), 80 mg/kg body weight - H & E x 400

Fig.33 - Kidney streaks of haemorrhage (A), massive desquamation of tubular epithelial cells (B), 80 mg/kg body weight - H & E x 100

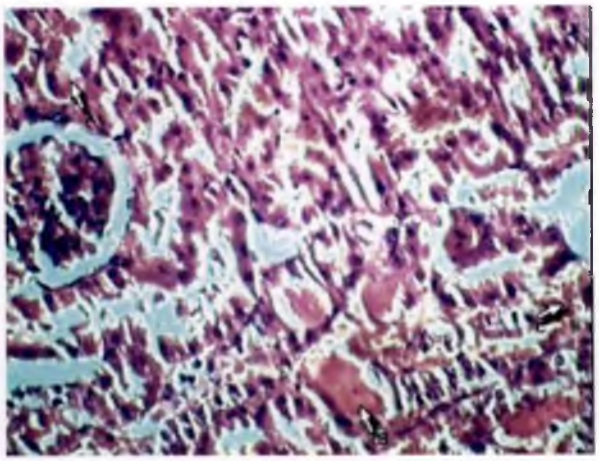


Fig. 34

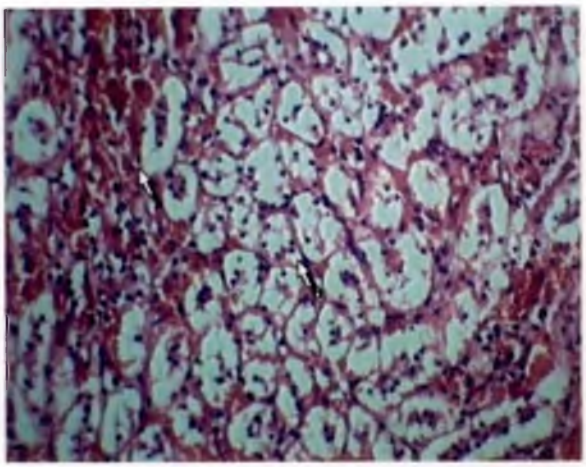


Fig. 35

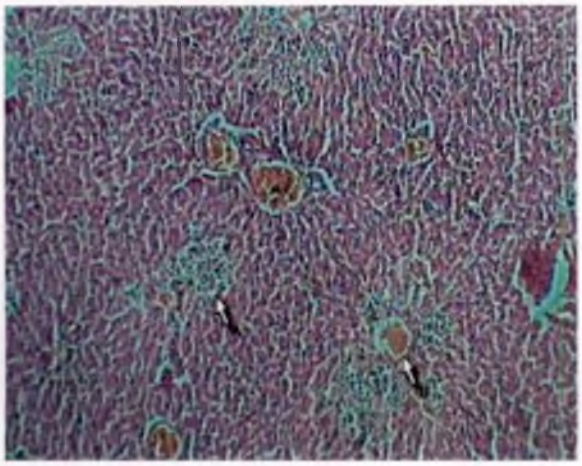


Fig.36

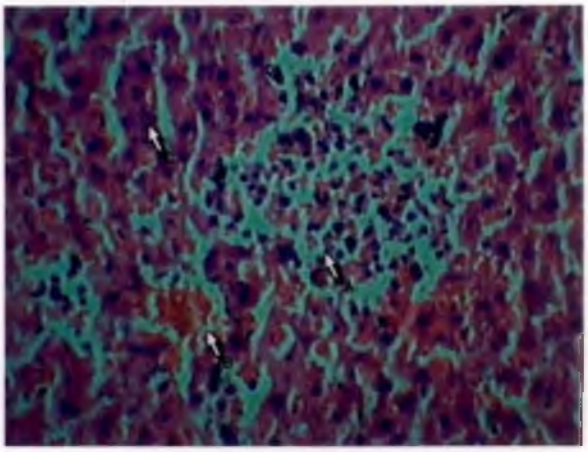


Fig. 37

Fig. 34 - Kidney - Vacuoles in cytoplasm of tubular epithelial cells (A), desquamation and hyalinization of epithelial cells (B), haemorrhage (C), 80 mg/kg body weight, H & E x 400

Fig. 35 - Kidney - Inter tubular haemorrhage (A), necrosis and denudation of tubular epithelial cells in medulla (B), 80 mg/kg body weight, H & E x 400

Fig.36 - Liver - Focal areas of necrosis (A), central venous congestion (B), 80 mg/kg body weight, H & E x 100

Fig. 37 - Liver - Focal areas of necrosis with mononuclear cell in filtration (A), sinusoidal congestion (B), massive degeneration of hepatocytes (C), 80 mg/kg body weight, H & E x 400

Fig. 38

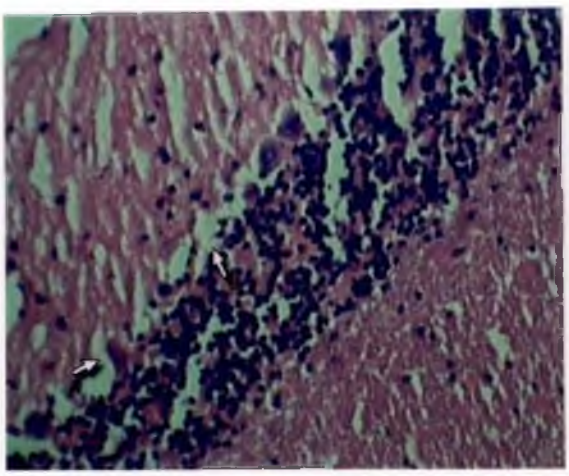


Fig. 39

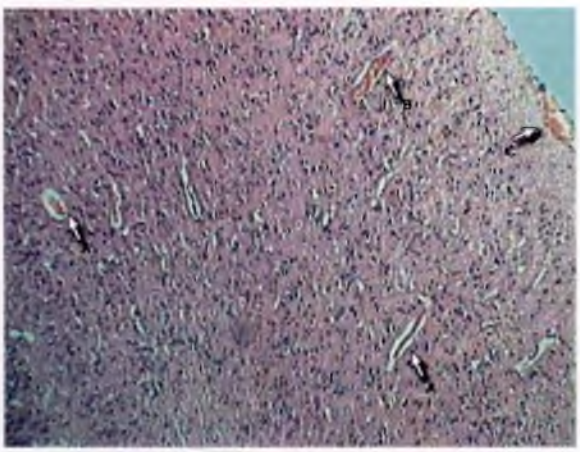


Fig. 40

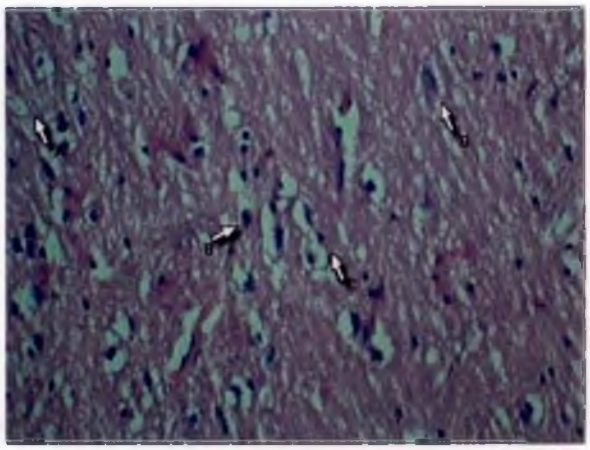


Fig. 41

Fig. 38 - Lungs - Congestion (A), extensive haemorrhage (B), oedema (C), 80 mg/kg body weight - H & E x 400

Fig. 39 - Cerebellum - Shrinkage (A) and loss of Purkinje cells (B), 120 mg/kg body weight - H & E x 400

Fig. 40 - Brain - Perivascular vacuolation (A), congestion in brain (B), congested meninges (C), 120 mg/kg body weight - H & E x 100

Fig. 41 - Brain - Spongiosis of white matter (A), perineuronal vacuolation (B), gliosis (C), neuronal necrosis, 120 mg/kg body weight - H & E x 400

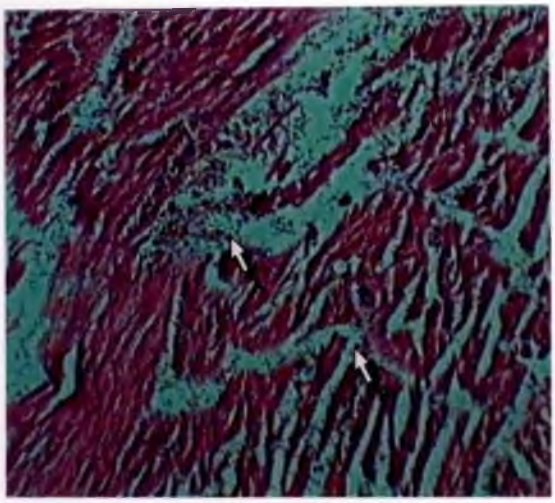


Fig. 42

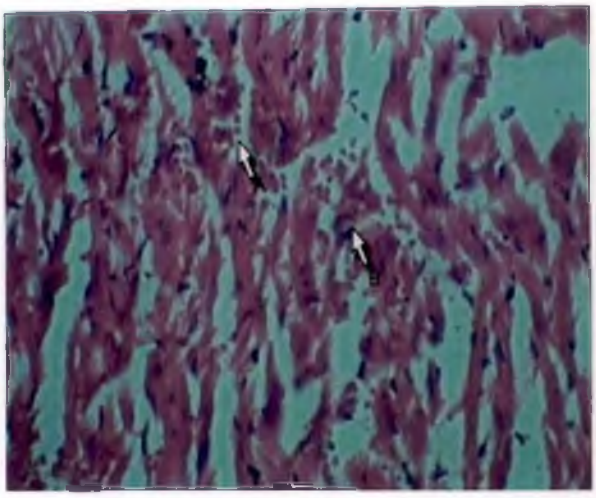
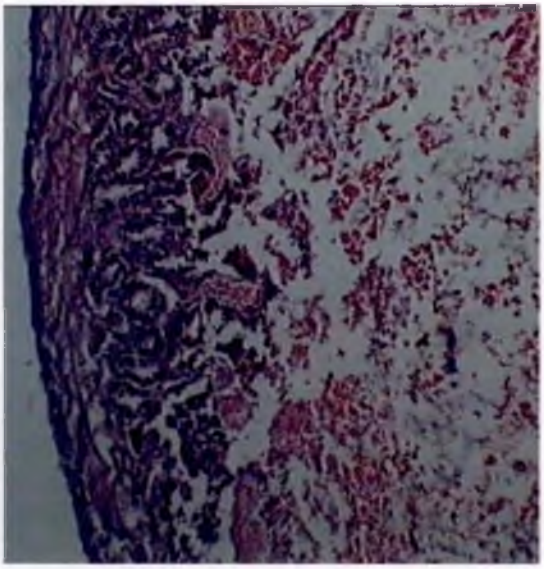


Fig.43



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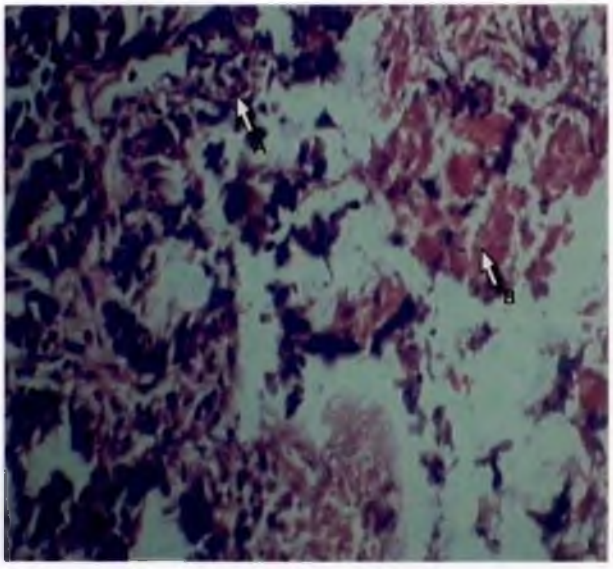

Fig. 45

Fig. 42 - Heart - Haemorrhage (A), myolysis (B), 120 mg/kg body weight - H & E x 100

Fig.43 - Heart - Haemorrhage (A), myolysis (B), 120 mg/kg body weight - H & E x 400

Fig.44 - Intestine - Extensive necrosis and erosion of intestinal mucosa, 120 mg/kg body weight - H & E x 100

Fig. 45 - Intestine - Infiltration of inflammatory cells (A), necrosed epithelium (B), 120 mg/kg body weight - H & E x 400

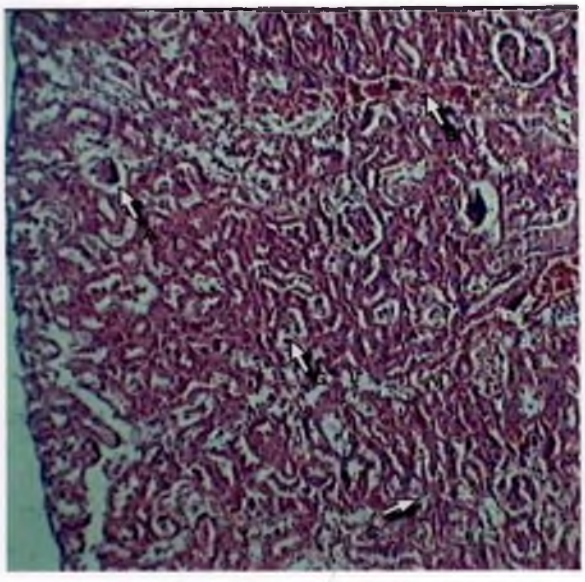

Fig. 46

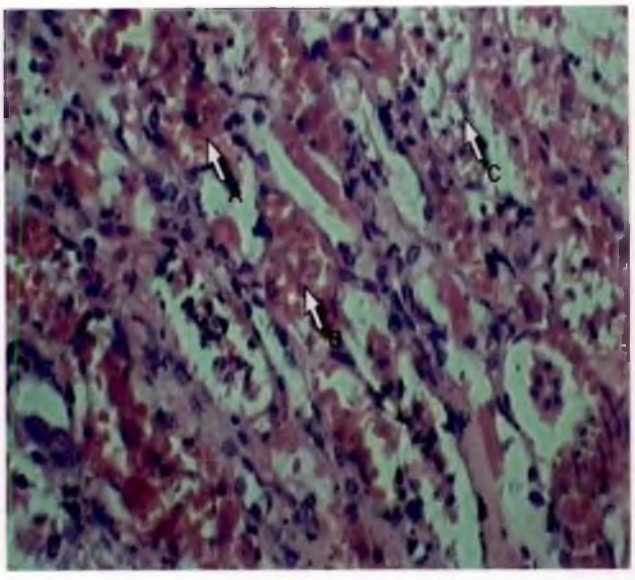

Fig. 47

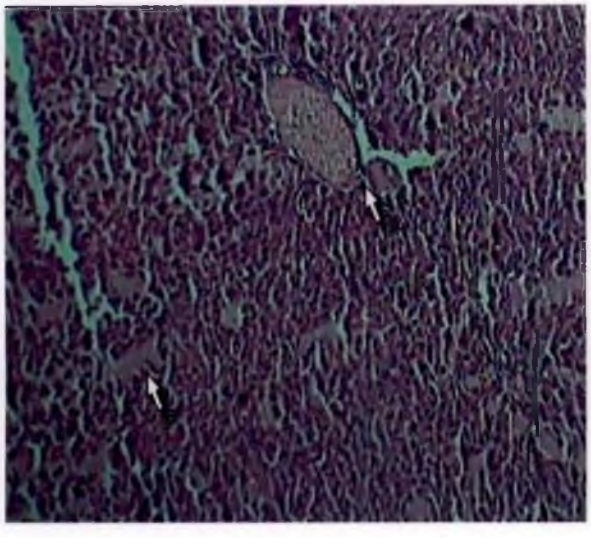

Fig. 48

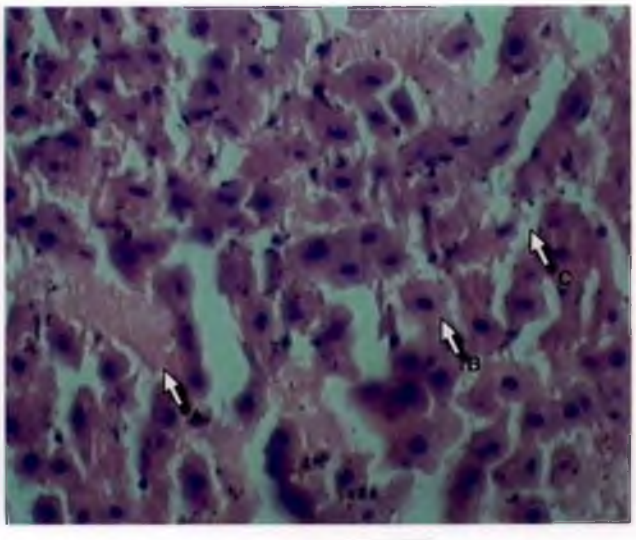

Fig. 49

Fig. 46 - Kidney - Glomerular atrophy (A), haemorrhage (B), necrosis (C), 120 mg/kg body weight - H & E x 100

Fig. 47 - Kidney - Intertubular haemorrhage (A), congestion (B), necrosis and desquamation of tubular epithelial cells, 120 mg/kg body weight - H & E x 400

Fig. 48 - Liver - Central venous congestion (A), lymph stasis (B), 120 mg/kg body weight - H & E x 100

Fig. 49 - Liver - Lymph stasis (A), swollen hepatocytes (B), dissociation of hepatic cords (C), 120 mg/kg body weight - H & E x 400

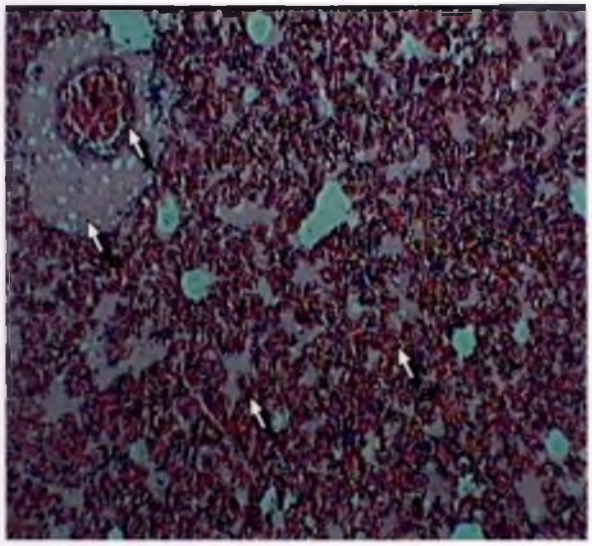

Fig. 50

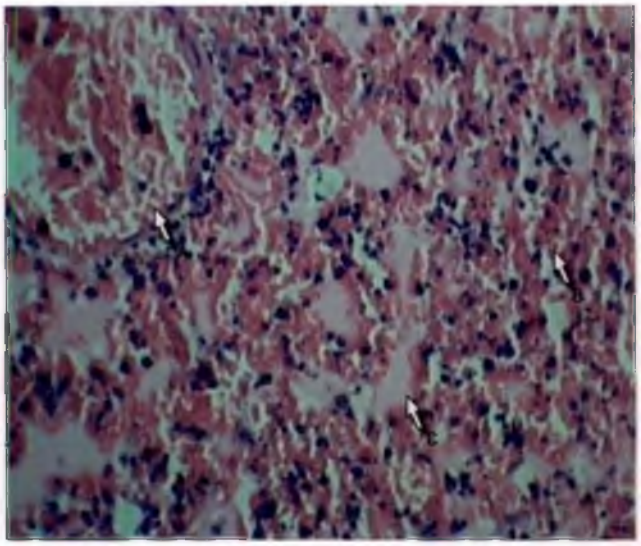

Fig. 51

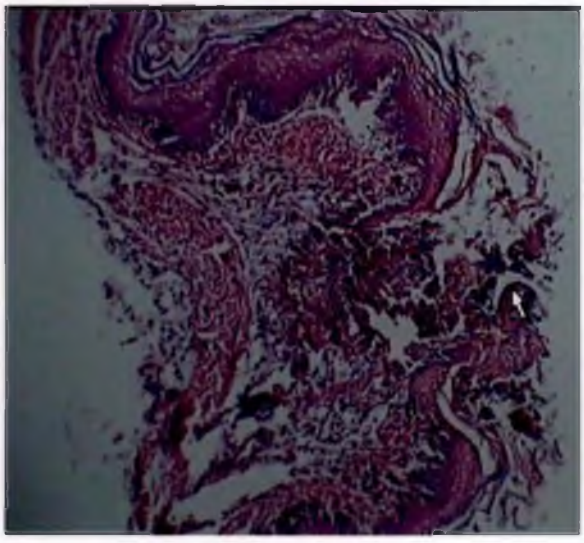

Fig. 52

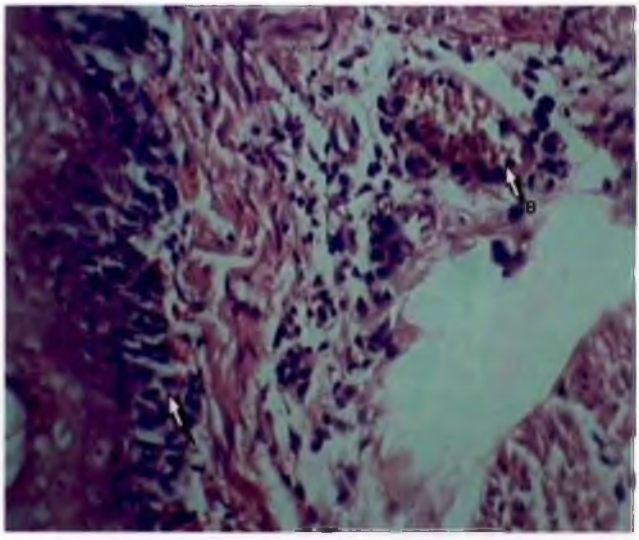

Fig.53

Fig. 50 - Lungs - Pulmonary congestion (A), perivascular oedema (B), oedema (C), extensive haemorrhage (D), 120 mg/kg body weight - H & E x 40

Fig. 51 - Lungs - Pulmonary congestion (A), extensive haemorrhage (B), oedema (C), 120 mg/kg body weight - H & E x 400

Fig. 52 - Stomach - Superficial erosion of epithelium (Arrow), crowning of the area by RBC and lysed cells, 120 mg/kg body weight - H & E x 100

Fig.53 - Stomach - Focal proliferation of basal cells of epithelium (A), congestion (B), 120 mg/kg body weight - H & E x 400

Discussion

5. DISCUSSION

Pyrethroids are among the most potent and effective insecticides available. In consonance with the other compounds of the pyrethroid family, cypermethrin exhibits good insecticide action and low mammalian toxicity. In view of this, it is widely used in agriculture, homes and public health programs to control a variety of insects. A number of studies have demonstrated the acute and chronic effects of cypermethrin on various animals. Inherent toxic potential can be high, but this is limited as it is rapidly detoxified via ester hydrolysis in the blood and liver (Ray and Forshaw, 2000). Since cypermethrin has a wide range of LD_{50} values in rats, the study of subchronic effect of different doses of cypermethrin in rats is significant. Effects of cypermethrin depend on dose, length and frequency of exposure. Effects also depend on the health of the individual and environmental factors (NTPN, 1998). This study is therefore conducted to evaluate the clinical signs, gross pathology, histopathology, haematological and biochemical effects of cypermethrin intoxication in rats.

5.1 BODY WEIGHT

Rats intoxicated with cypermethrin did not show any significant difference in body weight as compared with the control animals. The animals of the vehicle treated group showed a significant increase in the body weight as compared with the untreated ones. In the case of the cypermethrin intoxicated groups, although vehicle produced a weight gain, it could be nullified by cypermethrin intoxication. It can be infered that cypermethrin could bring about a reduction in the weight gain in intoxicated groups. Haratym (2002) observed reduction in body mass gain in cypermethrin intoxicated mice. A statistically significant reduction in the body weight of cypermethrin intoxicated rats was reported by Aldana *et al.*, (1998) where as Varshneya *et al.*,(1992), Luty *et al.*, (1998), Sayim *et al.*, (2005) and Yavasoglu et al.,(2006) observed no alteration in the body weight in rats intoxicated with cypermethrin at different doses. In rabbits, cypermethrin intoxication produced a

reduction in body weight gain (Lakkawar *et al.*, 2004). Hassan *et al.*, (1988) opined that pesticide treatment could bring about little effect on the body weight and feed efficiency of growing rabbits.

5.2 HAEMATOLOGICAL PARAMETERS

Haematological parameters of animals in groups I to IV are evaluated. As the animals in group V (highest dose group) died within one week of trial, haematological parameters could not be assessed.

5.2.1 Erythrocyte Parameters

There was a dose related reduction in haematological values like total erythrocyte count, haemoglobin concentration and packed cell volume in cypermethrin intoxicated rats. The reduction in values of TEC, PCV and haemoglobin concentration indicate depressed erythropoiesis. Reductions in the erythrocyte count, PCV and haemoglobin concentration were observed by Manna *et al.*, (2004b) and Sayim *et al.*, (2005) in their trial of cypermethrin intoxication in rats. The same observation was made by Hassan *et al.*, (1988) in growing male rabbits, Patel *et al.*, (1999) in crossbred cow calves and Haratym (2002) in mice after intoxication with various doses of cypermethrin. Madsen *et al.*, (1996) and Luty *et al.*,(2000) could not find any changes in the erythrogram after cypermethrin intoxication in Swiss mice and rats respectively. Manna *et al.*, (2005) observed decrease in PCV and haemoglobin concentration in a study of repeated dose toxicity of deltamethrin in rats.

5.2.2 Leukocyte Parameters

Total leukocyte count (TLC) and differential leukocyte count (DLC) showed significant variations in the intoxicated animals. Total leukocyte count showed a significant reduction in the intoxicated animals as compared with the control animals. Differential leukocyte count revealed significant increase in the lymphocyte count but

a significant reduction in the neutrophil count. The reduction in the leukocyte count can be considered as an indication of immunosuppressive effect of cypermethrin. A statistically significant increase in the number of leukocytes along with an increase of monocytes and lymphocytes was reported by Luty *et al.,* (2000) in Swiss mice in a study of sub acute toxicity of orally applied α cypermethrin. Haratym (2002) observed that lower doses of α cypermethrin produced an increase in the number of leukocytes in peripheral blood of male mice, but no statistically significant differences were noted in the percentage of lymphocytes and neutrophils in the peripheral blood of mice poisoned with α cypermethrin. Manna *et al.*, (2004b) observed a decrease in the lymphocyte and monocyte counts but an increase in neutrophil count in rats intoxicated with cypermethrin repeatedly. In rabbits, dimethoate and decamethrin produced a marked elevation in the total leukocyte count characterized by an increase in neutrophil count but a decrease in the lymphocyte, monocyte, basophil and eosinophil counts (Hassan *et al.*, 1988). In calves, sub acute toxicity of cypermethrin produced no effect on the TLC values whereas the number of lymphocytes was decreased but a slight increase in neutrophils was observed by Patel *et al.*, (1999). Manna *et al.*, (2005b) in a study on repeated dose toxicity of deltamethrin in rats observed decreased counts of lymphocyte, monocyte and eosinophil where as basophil count was increased significantly. Garg *et al.*, (1997) found that fluvalinate significantly altered TLC and DLC in sub acute toxicity.Varshneya *et al.*, (1992) observed a significant reduction in total leukocyte count at the highest dose of cypermethrin in rats. A significant decrease in the absolute WBC count without any change in the differential count was reported in rats in cypermethrin toxicity by Institoris *et al.*, (1999b).

5.3 BIOCHEMICAL PARAMETERS

A marked elevation of ALT and AST was found in the serum of cypermethrin intoxicated animals. But GGT levels in serum were found to be unchanged. Though cypermethrin toxicity affects most of the soft tissues in the body, bile ducts are not

damaged and hence the GGT values are not altered in the present study. The serum total protein content and albumin content were found to have a significant reduction in the intoxicated animals in the present study. Liver damage produced by cypermethrin could be the reason for reduction in serum protein levels. Muthuviveganandavel *et al.*, (2008) observed augmented ALT activity but declined GGT activity in serum of cypermethrin intoxicated male rats. Patel *et al.*, (2001) found that cypermethrin intoxication could produce a significant elevation in the transaminase (AST and ALT) activities in the serum of crossbred calves, but Kaur and Sandhu(2000) observed a significant decrease in the plasma AST and ALT activities in the cypermethrin intoxicated buffalo calves. Study of effects of cypermethrin on the hepatic function revealed statistically significant increase in serum AST and ALT, but a reduction in the albumin content although serum proteins remained unchanged (Aldana *et al.*, 1998). Manna *et al.*, (2004a&b) reported a significant elevation in serum AST and ALT in studies of single dose and repeated dose toxicities of α cypermethrin in rats. Cypermethrin intoxication in chickens revealed reduction in total proteins and albumin in serum (Khurana *et al.*, 1996) but no change in serum AST and ALT activities (Anwar, 2003). A significant elevation of serum AST and ALT was reported in repeated dose deltamethrin toxicity in rats. All these observations suggest that biochemical alterations induced by cypermethrin toxicity could be largely attributed to its pathological effects on the liver.

5.4 CLINICAL SIGNS

The clinical sign observed in the low dose group was loss of body weight during the first few days of intoxication. Later the animals adapted and gradually their body condition improved. Another sign noticed during the early days of intoxication was gradual development of hind limb extensor tone followed by recovery. Other signs noticed were increased startled response, salivation and somnolence. The medium dose group exhibited signs such as burrowing behavior,

increased startled response, salivation, somnolence, seizures and gradual development of hind limb extensor tone. Finally choreoathetosis developed and the animal became recumbent, showing laboured breathing, gasping and death. The highest dose group animals exhibited increased startled response, seizures, somnolence and salivation initially, but later showed loss of righting reflex, lateral recumbency, laboured breathing, gasping and death. Similar signs were observed by Aldana *et al.*, (1998) and Manna *et al.*, (2004b) in cypermethrin toxicity studies in rats at different dose levels. Cypermethrin, a cyanopyrethroid, produce type II syndrome or choreoathetosis/ salivation (CS) syndrome in rodents characterized by signs such as initial pawing and burrowing behavior, salivation, seizures, increased startled response and abnormal locomotion involving hind limbs (Garg, 2006). All these suggest that cypermethrin primarily affects the nervous system and the primary neurotoxic target sites are the voltage dependent sodium channels in excitable membrane. The prolonged sodium current induced by the pyrethroids results in pronounced repetitive activity which is manifested as CS syndrome (Vijverberg and Bercken, 1990). Near lethal doses can give rise to axonal degeneration in the peripheral nerve closely resembling Wallarian degeneration, but this effect is inherently reversible and is seen only at dose levels that produced prolonged and severe motor signs. The longer current prolongation can cause in coordination, choreoathetosis, seizures and direct effect on skeletal and cardiac muscle and salivary glands (Ray and Forshaw, 2000).

5.5 MORTALITY PATTERN

The group treated with 120mg/kg body weight (group V) of cypermethrin showed 100% mortality within 3-7 days. The medium dose group given 80 mg/kg body weight of cypermethrin, showed 75% mortality and most of the deaths occurred within 6-10 days. Manna *et al.*, (2004b) reported that LD_{50} of α cypermethrin is 145 mg/kg body weight. Animals of the lowest dose group and control group did not show any mortality indicating that the mortality is due to cypermethrin toxicity.

5.6 PATHOANATOMICAL STUDIES

5.6.1 Gross pathology

The lowest dose group showed bloat, congestion of the lungs, brain and gastric mucosa with mild degenerative changes in the liver and kidneys. The postmortem lesions of medium and highest dose group animals that died during the experiment were bloat, pulmonary congestion with extensive pulmonary haemorrhage, congestion of the brain and heart and degenerative changes in the liver and kidneys. Congestion of the lungs,brain,liver and gastric mucosa were observed in medium dose group animals that survived the experiment. Manna *et al.*, (2004a&b) observed bloat, haemorrhages in the stomach, intestine and lungs in both single dose and repeated dose toxicity studies of cypermethrin in rats. No gross morphological changes were observed in visceral organs by Luty *et al.*, (1998) in a study of toxicity of dermally applied cypermethrin in rats. In crossbred calves, cypermethrin intoxication produced congestion and / or haemorrhages on auriculoventricular groove of heart, lungs, mucosal surface of intestine, cortex of kidney and brain (Patel *et al.*, 2000b). The observations in repeated dose toxicity of deltamethrin like bloat, haemorrhage in stomach, intestine and lungs of rats was in line with the findings of Manna *et al.*, (2005b).

5.6.2 Histopathology

5.6.2.1 Brain

Brain of group III animals revealed gliosis, perineuronal vacuolation, perivascular vacuolation, neuronal degeneration and congestion. In animals treated with 80 mg/kg body weight of cypermethrin, degenerative changes were characterized by shrinkage of the neurons, perineuronal vacuolation, moderate vacuolation of the white matter, perivascular vacuolation and moderate gliosis in the cerebrum. The highest dose group namely group V, revealed discontinuity and shrinkage of Purkinje cells in cerebellum. The other lesions were congestion in the

meninges and cerebrum, perineuronal vacuolation, perivascular vacuolation and gliosis. In the cerebellum, pyknosis of Purkinje cells and disappearance of some of the cells were observed by Luty *et al.*, (1998) in cypermethrin intoxicated rats. Pyknosis of neurons was observed by Latuszynska *et al.*, (2001) and Sayim *et al.*, (2005) in their studies of cypermethrin intoxication in rats. Congestion and degenerative changes in the brain were reported by Patel *et al.*, (2000b) and Muthuviveganandavel *et al.*, (2008) in calves and rats respectively. Manna *et al.*, (2004a &b) reported congestion and haemorrhages in the brain of rats intoxicated with single and repeated doses of cypermethrin. The results of the present study are in agreement with the results of various other studies conducted previously to evaluate toxic effects of cypermethrin through various routes in different species of animals.

5.6.2.2 Spinal cord

The spinal cord lesions observed in group III animals were large neuronal degeneration, perineuronal vacuolation and vacuolation of the white matter. However no lesions were noticed in the of group IV and V animals. Vijverberg and Bercken (1990) demonstrated histopathological lesions in the nerves in rats, mice and hamsters receiving lethal or near lethal oral doses of pyrethroids. The lesions have been observed mostly in the peripheral nerves and occasionally in the spinal cord and brain. Lesions were observed only in a fraction of the treated animals.

5.6.2.3 Liver

Varying degrees of degenerative changes were the most consistent lesion observed in the liver of animals of all the treatment groups. Vascular changes were also evident in the liver sections. In some areas, changes varied from degenerative to necrotic. In the liver of group III animals, Kupffer cell hyperplasia was pronounced. These findings are in agreement with those observed in cypermethrin toxicity in rats by Muthuviveganandavel *et al.*, (2008), Yavasoglu *et al.*, (2006), Manna *et al.*, (2004a &b) and Luty *et al.*, (1998). All have done experiments in albino rats, but the

dosage and route of administration of cypermethrin were different. In crossbred cow calves, cypermethrin intoxication produced moderate congestion of the sinusoids and hepatic vasculature and the hepatocytes showed tiny vacuoles along with increased granularity in the cytoplasm of liver (Patel *et al.*, 2000b). In a nut shell, cypermethrin produced varying degrees of degenerative changes in all species of animals intoxicated irrespective of the dose and route of administration.

5.6.2.4 Oesophagus

The oesophagus in all intoxicated groups revealed no lesions. It can be concluded from this observation that cypermethrin does not produce any pathological effects on the oesophagus.

5.6.2.5 Stomach

In the case of the stomach, the highest dose group (group V) revealed superficial erosion with presence of RBC and lysed cells in the non glandular portion of the stomach. The lowest and medium dose group (group III and IV) did not reveal any lesions. Manna *et al.*, (2004a &b) observed desquamation and necrosis of the epithelium of stomach of rats intoxicated with single and repeated doses of cypermethrin. The result of the present study indicates that only high dose of cypermethrin could produce gastric irritation.

5.6.2.6 Intestine

Diffuse hyperplasia of the goblet cells and inflammatory cell infiltration into the villous epithelium of intestine were observed in group III and group IV whereas diffuse necrosis of the villous epithelium was observed in group V. Patel *et al.*, (1997) reported erosive enteritis in cypermethrin intoxicated calves and laboratory animals. From these observations it can be concluded that both high and low doses of cypermethrin could irritate the intestinal epithelium.

5.6.2.7 Lungs

In group III animals, the lungs revealed varying degrees of pulmonary congestion and oedema while those of the group IV and group V exhibited pulmonary oedema, varying degrees of pulmonary congestion and haemorrhages. These are the lesions observed in most of toxicity studies with cypermethrin. Patel *et al.*, reported mild to moderate congestion, oedema, haemorrhage and focal areas of emphysema in cypermethrin intoxicated calves. Manna *et al.*, (2004a &b) reported the same lesions in rats intoxicated with single and repeated doses of cypermethrin. These observations indicate that cypermethrin does have cytotoxic effects in the lungs.

5.6.2.8 Kidney

Varying degrees of degeneration extending to necrosis was observed in the tubular epithelial cells of animals of all treatment groups. Other lesions encountered in group III were congestion and diffuse atrophy of the glomeruli. In the medium dose and high dose groups animals, apart from lesions mentioned above, multiple haemorrhages in both cortical and medullary tubules besides in interstitium were evident. Luty *et al.*, (1998) observed parenchymatous degeneration in the tubular epithelium of rats. Latuszynska *et al.*, (1999) observed infiltration of mononuclear cells between proximal tubules and around blood vessels in rats intoxicated with cypermethrin and chlorpyrifos. Patel *et al.*, (2000b) reported vacuolation of cytoplasm and rarefaction of the parenchyma of tubular epithelial cells of kidneys along with intense congestion in cypermethrin intoxicated calves. Lakkawar *et al.*, (2004) found hyalinization of tubular epithelium of kidneys in rabbit in cypermethrin toxicity. Destruction of the tubular epithelium was observed by Muthuviveganandavel *et al.*, (2008).

5.6.2.9 Heart

Lowest dose group (group III) revealed no microscopic lesions in the heart. Haemorrhage and congestion were observed in the myocardium of group IV and V animals while group V animals exhibited focal myolysis as well. Patel *et al.*, (1999) found moderate congestion and loss of striation in myocardial fibers of the crossbred calves and laboratory animals intoxicated with cypermethrin. Muscle bands were observed to be in patches and blood vessels were found to have ruptured in the myocardium of rats in cypermethrin toxicity. (Muthuviveganandavel *et al.*, 2008).

The systematic investigation conducted brought to the light that apart from its neurotoxic action, cypermethrin, which is considered to be a safer insecticide, posses hepatotoxic, nephrotoxic, enterotoxic, cardiotoxic and pneumotoxic actions as well. Haematological parameters revealed that cypermethrin could affect erythropoietic system. Leucopenia produced as a result of toxicity indicates its immunosuppressive effects. Biochemical parameters revealed that the pathological effects could be attributed to its detrimental effects on the liver, the major detoxifying organ of the body, as well as other visceral organs. Histological studies revealed multisystemic effects of cypermethrin. High dose of cypermethrin even produced necrosis and extensive haemorrhage in major organs of the body like liver, lungs and kidneys. The results suggest that biochemical effects rather than histological alterations due to damage at sub cellular level could be the reason for toxicity. To conclude strict guidelines need to be enforced in order to minimize the indiscriminate use of insecticides thereby avoiding hazards to animals, environment and ultimately to the human population.

Summary

6. SUMMARY

The experiment was designed to study the pathomorphological changes of cypermethrin induced toxicity in rats on the nervous and digestive system along with its effects on biochemical and haematological parameters.

Adult Sprague Dawley rats weighing approximately 200 g were used for the study. The animals were divided into five groups randomly. Group III to V were intoxicated with cypermethrin orally at the dose level of 40mg/kg, 80mg/kg and 120 mg/kg of body weight respectively for 21 days. Gingely oil was used as the vehicle for administration of cypermethrin. The parameters observed included clinical signs, gross pathology, histopathology, haemogram and biochemical parameters like enzyme estimation and serum protein estimation. Gross pathology included postmortem lesions and weight variation in animals at the beginning of the experiment and at the end of the experiment. Histopathology of brain, spinal cord, oesophagus, stomach, intestine, liver, kidney, lungs and heart were evaluated. Packed cell volume, haemoglobin concentration, total erythrocyte count, total leukocyte count and differential leukocyte count were evaluated at weekly intervals. Serum levels of alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transferase were compared at the beginning and at the end of the experiment. Total protein, albumin and globulin content in serum were estimated and from it A: G ratio was calculated. Since all the animals in highest dose group died within one week, haematological and biochemical parameters of this group could not be assessed.

The study revealed a dose related increase in mortality. In groups treated with high dose and medium dose of cypermethrin, 100% and 75% mortality were observed respectively.

Clinical signs observed included burrowing behaviour, salivation, somnolence, gradual development of hind limb extensor tone, seizures and increased startled response. When symptoms progressed, the animal went into lateral recumbency, showed laboured breathing, gasping and death. The symptoms were shown only by animals in groups IV and V. All animals in high dose group died within one week. Group III animals exhibited only a loss of weight and somnolence.

The mean body weight of the intoxicated animals did not show any variation as compared with the control animals, but showed a significant variation in comparison with the vehicle treated animals.

Haematological parameters such as packed cell volume, haemoglobin concentration, total erythrocyte count and total leukocyte count showed a dose dependent reduction. The differential leukocyte count revealed lymphocytosis and neutropenia in cypermethrin intoxicated groups.

Biochemical parameters such as levels of AST and ALT revealed significant increase in the animals of the intoxicated groups. But GGT level did not show any variation in the treatment groups. The total serum protein, albumin and globulin levels were found to be decreased in these groups.

Gross pathological changes observed in treatment groups were bloat, congestion of the lungs, brain and heart besides degenerative changes in the liver and kidneys. Animals of groups IV and V showed extensive haemorrhages in the lungs.

Degenerative changes in hepatocytes with subsequent necrotic changes and central venous congestion were observed in animals of groups III and IV. The liver of animals intoxicated with high doses of cypermethrin revealed dilatation of the sinusoids with lymph stasis and central venous congestion.

In group III animals, lesions in the brain were gliosis, perineuronal and perivascular vacuolation. In group IV animals cerebral lesions included shrinkage of neurons, perineuronal vacuolation, perivascular vacuolation, spongiosis of the white matter and moderate gliosis. In group V, discontinuity and shrinkage of Purkinje cells in the cerebellum, perineuronal vacuolation, perivascular vacuolation, gliosis and congestion in brain and meninges were observed.

In animals intoxicated with 40 mg/kg body weight of cypermethrin, the spinal cord revealed degeneration of large neurons, perineuronal vacuolation and white matter vacuolation. Lesions in spinal cord were absent in groups treated with 80mg and 120mg/kg body weight of cypermethrin.

There were no lesions in the oesophagus of animals of all the intoxicated groups. In the stomach of high dose group, superficial erosion of the gastric mucosa along with presence of erythrocytes and lysed cells were observed. No significant changes could be observed in the stomach of groups treated with 40mg and 80 mg/kg body weight of cypermethrin.

Intestines showed diffuse hyperplasia of the goblet cells and inflammatory cell infiltration into lamina propria and submucosa in the low and medium dose group. In the highest dose group, diffuse necrosis of villous epithelium was observed.

Varying degrees of pulmonary congestion and oedema were observed in the lungs of all treatment groups. Apart from this, the lungs of animals of the medium and high dose groups revealed extensive haemorrhages into the alveoli.

As in the case of the other organs evaluated, degenerative changes were predominant in the kidneys of the treatment groups. Intensity of changes in the

kidneys varied depending on the dosage. Tubular epithelial cells were the most affected.

Intermuscular haemorrhage, congestion and myolysis were the lesions observed in heart of animals of groups administered 80 mg/kg and 120 mg/kg body weight cypermethrin while those of the the lowest dose group did not reveal any lesion.

The result of this study revealed that pyrethroids can no longer be considered as nontoxic to mammals and hence underscore the need for the judicious use of these compounds as insecticides in domestic animals.

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GASTROINTESTINAL AND NEUROTOXIC EFFECTS OF CYPERMETHRIN IN RATS

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ABSTRACT

The present study entitled 'Gastrointestinal and neurotoxic effects of cypermethrin in rats' was undertaken to investigate the potential toxic effects of cypermethrin in rats with particular emphasis on its effect on the nervous and digestive system.The clinical signs, gross pathology, histopathology, haematology and biochemical parameters were analysed to study the effects.

Cypermethrin was found to be neurotoxic and gastroenterotoxic at the given dose levels. Oral administration of cypermethrin in medium and high doses produced nervous signs in animals. However cypermethrin did not cause any significant variation in the body weight of animals.

All haematological parameters evaluated showed a dose dependent reduction in its value. Biochemical parameters assessed revealed hepatotoxic and multisystemic effects of cypermethrin.

Gross lesions observed in the intoxicated groups were bloat, congestion of lungs, heart, brain, pulmonary haemorrhage and degenerative changes in liver and kidneys.

On histopathological examination, cypermethrin was found to be neurotoxic, hepatotoxic, enterotoxic, pneumotoxic, cardiotoxic and nephrotoxic as evidenced by varying degrees of degeneration and necrosis in various organs examined. Effects on all organs were mild to moderate degenerative changes at the low dose level. Medium and high dose intoxicated groups revealed necrotic changes, extensive haemorrhages, congestion in organs like liver, kidney and lungs apart from the changes observed in low dose group animals. Haemodynamic disturbances were manifested in the forms of congestion and haemorhages in lungs, heart, liver and kidneys. The

histopathological evaluation revealed no cypermethrin induced toxic effects on the oesophagus. Spinal cord lesions were observed only in the lowest dose group. Brain lesions were only degenerative changes. These findings suggested that biochemical effects rather than structural changes were involved in toxicity.