STUDIES ON THE PATHOLOGICAL EFFECTS OF FIPRONIL AND THEIR AMELIORATION BY CURCUMIN IN RATS

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DECLARATION

I hereby declare that this thesis entitled **"PATHOLOGICAL EFFECTS OF FIPRONIL AND THEIR AMELIORATION BY CURCUMIN 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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Introduction

1. INTRODUCTION

Pesticides have a major role in boosting the agricultural production as well as controlling ectoparasites in domestic animals. Among pesticides, insecticides represent a large proportion of the compounds synthesized by the chemical industry for agricultural and animal pests. Older insecticide families are carbamates, organophosphates, organochlorines and pyrethroids. Recently, they are limited in application due to increasing resistance development and implication in potential health hazards to livestock, wildlife, fishes, birds, and human beings. Hence, it seems that older pesticides would be replaced by phenylpyrazoles and neonicotinoids family in future. Among them are the most widely used insect growth inhibitors marketed under different names such fipronil, imidacloprid and selamectin etc.

Fipronil, a highly effective second generation phenylpyrazole insecticide was introduced commercially in 1993. Fipronil is herbicide and acaricide commonly used as topical agent against ectoparasites in pets. Recent years, it is widely used as an insecticide in agricultural practices. The technical grade fipronil is a white powder with a mouldy odour and sparingly soluble in water. Fipronil is a potent non competitive gamma-aminobutyric acid (GABA) inhibitor and potentially inhibits glutamate-activated chloride channels (GluCls), which are present in invertebrates such as insects, but not in mammals. Insect death is caused by hyper-excitation, convulsions and paralysis by contact and ingestion. Five degradation products are found in the environment such as fipronil-sulfone, fipronil-sulfide, fipronil desulfinyl, fipronil-amide, and fipronil-detrifluoromethylsulfinyl. Fipronil-sulfone is twenty times more toxic than fipronil. Fipronil is effective against insects that are resistant to other agents such as pyrethroids, organophosphates, and carbamates. Fipronil residues have been detected in drinking water, maize, Chinese cabbage and milk. Indiscriminate use of fipronil in animals will have its adverse effects on the various systems. Toxicological studies have shown that fipronil binds to mammalian $GABA_A$ and

$GABA_C$ receptors. Fipronil is rapidly metabolized and its residues widely distributed in the tissues, particularly brain, liver, kidney, fat and feces.

Fipronil can modify the metabolism of several endogens and xenobiotic compounds by the modulation of cytochrome P450 enzymes and particularly by the induction of CYP3A4 isoenzymes in human hepatocytes. In rodents, cytochrome P450 modulation promotes the development of thyroid tumours through the disruption of hepatic metabolism and excretion of thyroid hormones (Hurley *et al.*, 1998). In the same way, fipronil can induce adverse reproductive effects by altering cytochrome-dependent progesterone and estradiol metabolism in female rats (Ohi *et al.*, 2004). However, endocrine disruptions observed in rats and humans are not systematically correlated and further investigations are necessary to determine the endocrine-disrupting activity of fipronil in mammals. But in humans, fipronil and its sulfone metabolite induce cytotoxicity in hepatocytes to a greater extent than endocrine distruption.

Indigenous herbal drugs have higher demand for ameliorating toxic compounds recently. *Curcuma longa* is one among them which posses anti-inflammatory, antioxidant (Daniel *et al.*, 2004), antibacterial, immuno-stimulant, antifungal, antiprotozoal, antimutagenic, antidiabetic, anticarcinogenic, antifibrotic, antiulcer, hypotensive, hepatoprotective (Park *et al.*, 2000) and hypocholestremic activities.

Curcuma longa (Turmeric) is a tropical plant native to southern and southeast Asia. It is a perennial herb belonging to the ginger family and measures up to one meter height with a short stem and tufted leaves. The part medicinally used are the rhizomes. The most active component in turmeric is curcumin, which may make up two to five percent of the total spice in turmeric. Curcumin (diferuloylmethane) derived from turmeric by ethanolic extraction, is a pure orange-yellow, crystalline powder insoluble in water.

Perusal of literature reveals paucity of information on the general toxic effects of fipronil. Comprehensive studies on the adverse effect of fipronil in suitable model become significant in this context. Hence this study is planned, and the results of which may yield reliable information on the toxicity which can help to manage adverse effects in accidental case of toxicity. The present study therefore envisaged to use rat as a model system to accomplish the following objectives:

1. Study the toxicopathological effects and hematobiochemical alterations of fipronil in rats:
2. Study the membrane damaging effects of fipronil.
3. Study the ameliorative effect of curcumin on the fipronil toxicity.

Review of Literature

2. REVIEW OF LITERATURE

A wide range of insecticides are intensively used against insects of plants and the ectoparasites on animals. Insecticides consist of a large variety of chemical agents having diverse chemical structure and biological activities. Fipronil is a broad spectrum phenylpyrazoles insecticide used to control agricultural pests, fleas, ticks, cockroaches and ants. Fipronil is one of the emerging ectoparaciticides in veterinary medicine, which possess potential insecticidal properties with low mammalian toxicity.

2.1 FIPRONIL

Fipronil was discovered by Rhone-Poulenc in 1993 under trade names 'Frontline' and 'Top spot'. Technical-grade fipronil contains 95.6 per cent fipronil. Fipronil {5-amino-1-[(2, 6-dichloro-4-trifluoromethyl)phenyl]-4-(trifluoromethyl-sulfinyl]-1H-pyrazole-3-carbonitrile} belongs to phenylpyrazole family. Fipronil was classified by world health organization into Class II moderately hazardous pesticide (United States Environmental Protection Agency, 1996). It was formulated as water dispersible granules (WG), micro granules (GR), flowable solid (FS), soluble concentrate (SC) and Ultra Low Volume (ULV) (Tingle *et al.*, 2003).

2.1.1 Half Life

Tingle *et al.* (2003) observed that fipronil was slowly degraded into amide, fipronil-desulfinyl and sulfone by oxidation, reduction and hydrolytic pathways respectively. In aerobic condition, the half life ($t_{1/2}$) of fipronil was 18-308 days where as under anaerobic condition it was 116-130 days.

2.1.2 Lethal Dose (LD_{50})

Fipronil was a moderately hazardous pesticide and the acute oral LD_{50} was 97 mg/kg body weight in rats. It was less toxic to mammals. Dermal absorption

in rats was less than one percentage after 24 hours and it had moderate dermal toxicity in rabbits. Fipronil was neurotoxic in both rats and dogs (World Health Organization, 1998).

Gunasekara *et al.* (2007) reported that oral LD_{50} of fipronil was 354 mg/kg body weight in rabbits. In mallard duck, pigeon, sparrow and bobwhite quail, lethal doses (LD_{50}) were 2150mg/kg, 2000mg/kg and 1120 mg/kg and 11.3 mg/kg body weight respectively.

2.1.3 Toxicokinetics

Hainzal and Casida (1996) observed the presence of desulfinyl fipronil in brain, liver and kidney for seven hours and in faeces for 24 hours after treatment with fipronil in rats.

Radio labeled fipronil [C^{14}] administered in dairy goats at dose of 0.05, two and ten ppm for seven days revealed excretion of fipronil mainly through faeces only. Retention rate was higher at two ppm compared to other doses while at ten ppm, milk had higher proportion of fipronil residues (Joint Meeting of FAO/WHO on Pesticidal Residues, 2001)

Fipronil and its metabolites were cleared by renal or hepatic mechanisms. Tissue concentrations were higher on seventh day with the highest levels in the fat and moderate levels in the adrenal gland, pancreas, skin, liver, kidney, muscle and thyroid gland in rats. Among fipronil metabolites fipronil-sulfone was the major residue in the tissues (Anon., 2004).

2.1.4 Mechanism of Action

Ikeda *et al.* (2001) showed the mechanism of fipronil interaction with the mammalian GABA system through whole-cell patch-clamp experiments by using rat dorsal root ganglion neurons (DRG) in primary culture. They observed that fipronil blocked GABA-induced currents slowly and reversibly and suggested that fipronil acted on $GABA_A$ receptor at closed ionic channel.

By whole-cell patch-clamp technique, Zhao *et al.* (2005) demonstrated that fipronil sulfone, a major metabolite of fipronil was a potent inhibitor of GABA receptors of rats and cockroaches.

2.1.5 Toxicity of Fipronil

2.1.5.1 Human Toxicity

Chodorowski and Anand (2004) observed headache, nausea, vertigo and weakness in a 50-year-old man exposured five hours to agricultural product of fipronil in the field. Symptoms developed after two hours of exposure and resolved spontaneously. They suggested that inhalation and dermal contact were the main route of exposure.

Sweating, nausea, vomiting, headache, abdominal pain, dizziness, agitation, weakness and tonic-clonic seizures were exhibited by humans due to accidental ingestion of fipronil. Clinical signs were generally reversible and resolved spontaneously (National Pesticide Information Centre, 2009).

2.1.5.2 Neurotoxicity

2.1.5.2.1 Neurotoxicity of Fipronil

Single dose of fipronil administered in rats at doses of 2.5, 7.5 and 25 mg/kg bodyweight in cornoil by gavage caused neurobehavioral signs such as convulsions, chewing, licking of lips and wet anogenital region at 25 mg/kg bodyweight in all animals of both the sexes at seventh hour of dosing (WHO, 2000).

In a 90 day study of toxicity, fipronil-desulfinyl administered in the diet to dogs at doses of 3.5, 9.5 and 35 ppm resulted in increased salivation, prostration, tremors, convulsions, noisy breathing, dyspnea, excessive barking, aggressiveness and irritability at 35ppm(Joint Meeting of FAO/WHO on Pesticidal Residues, 2001)

Dogs receiving fipronil-desulfinyl in the diet for a period of 28-days at doses of zero, 27, 80 and 270 ppm showed heavy mortality on fifth day at 80 and 270 ppm and the experiment was terminated. Dogs developed nervous signs such as clonic convulsion on tenth day at 27 ppm (Tingle *et al.*, 2003).

Dietary administration of technical grade fipronil at five milligram per kilogram body weight to beagle dogs for one year showed neurological signs such as body tremors, stiffened limbs, unsteady gait, lack of coordination, head nodding and muscle twitching at termination of study (Anon., 2004)

Rats administered with fipronil at doses of 50, 80, 126 and 200 mg/kg in corn oil showed heavy mortalities from four hours to three days after dosing at dose level of 80mg/kg. Other common signs were piloerection, hunched posture, abnormal gait and diarrhoea in both sexes. Decreased respiratory rate, pallor of extremities and ptosis were observed in all animals receiving more than 126mg/kg of fipronil (Fluoride Action Network, 2004)

Szegedi *et al.* (2005) observed that intragastric administration of fipronil in Sprague-Dawley rats at dose of 100mg/kg for a week resulted in increased amount of delta power or deep sleep on the first day and shifted the maximum of deep sleep to the dark phase on the second day. They also observed that fipronil increased neuronal excitability and its effect lasted slightly longer.

Stehr *et al.* (2006) studied the toxicological effect of fipronil on zebrafish embryos at nominal concentrations at or above 0.7mM (333 mg/l). Notochord degeneration, shortening of the rostral-caudal body axis, ineffective tail flips and uncoordinated muscle contractions were the features observed. They suggested that fipronil impairs the development of spinal locomotor pathways in fish by inhibiting a structurally related glycine receptor subtype.

Neurotoxic signs such as increased motor activity, excessive jumping, irritability to touch, compulsive biting and convulsions were observed in mice

administered with fipronil-desulfinyl at a dose of 30ppm (World Health Organization, 2007).

Lassiter *et al.* (2009) studied the compararive neurotoxicity of fipronil and chlorpyrifos in undifferentiated and differentiating neuronotypic PC12 cells by evaluating indices of cell replication, cell number, differentiation and viability. Fipronil reduced cell numbers in undifferentiated PC12 cells by inhibition of DNA and protein synthesis compared to chlorpyrifos. They suggested that fipronil was inherently a more potent disruptor of neuronal cell development than chlorpyrifos.

Exposure of fipronil and fipronil metabolites at a dose of 150 μM to *in vitro* intestinal epithelium for 24 hrs resulted in loss of barrier integrity, severe ATP depletion and loss of cell viability in the intestinal epithelium (Vidau *et al.*, 2009).

2.1.5.2.2 Neurotoxic Effects of Relative Insecticides

Dietary administration of endosulfan at dose levels of three milligram and six milligram per kilogram per day to both sexes of rats for 30 days revealed mild spontaneous motor activity and convulsion mostly in males on 15th day of administration whereas 30 per cent mortality was noticed in 30 days experiment at a dose of six milligram per kilogram per day (Paul *et al.*, 1995).

Hussain *et al.* (1996) reported that adult albino rats which received deltamethrin in formulations at dose of seven milligram per kilogram body weight for 15 days resulted significant reduction in mean body weight from 7th day onwards.

Kaul *et al.* (1996) observed that intraperitoneal administration of fenvalerate, a synthetic pyrethroid, in male rats for 45 days in doses of 100 and 200 mg/kg body weight/day induced hyper-excitability, tremors and convulsions. Tremors were observed after seven days and gradually reached maximum on 45th day. The symptoms were more marked in rats treated with 200 mg/kg body weight/day.

Parmer *et al.* (2003) observed that oral administration of lindane insecticide in rats at 2.5, five, ten and 15 mg/day for five days caused hyperexcitablity and convulsions on third day at dose of 15mg. They observed that lindane primarily targeted γ-aminobutyric acid (GABA) receptor ionophore complex.

2.1.5.3 Thyrotoxicity Effects

2.1.5.3.1 Thyrotoxicity of Fipronil

Hill *et al.* (1998) observed the new generation pesticide including fipronil was produced higher demand of thyroid hormone in rodents by T_3 and T_4 clearance in the liver. TSH of pituitary produced more amount of thyroid hormone by increasing the size (hypertrophy) and number (hyperplasia) of thyroid follicular cells to enhance hormone output.

Hurley *et al.* (1998) observed that fipronil enhanced hepatic thyroid hormone (T_4) metabolism and excretion in rats. Fipronil also increased iodide uptake in thyroid gland by higher activity of thyroid peroxidase. They also observed thyroid follicular tumors in male rats exposed to fipronil for two years at a dose range of 3.5-30.9mg/kg/day.

Oral administration of fipronil in male rats at a dose rate of 12.68 mg/kg/day in males and 16.75 mg/kg/day in females for 90 days resulted in the development of thyroid follicular cell tumour by disruption of the thyroid-pituitary status (WHO, 1998).

Hovda and Hooser (2002) reported that fipronil was classified into a group C (possible human) carcinogen.

2.1.5.3.2 Thyrotoxicity of Related Insecticides

Akhtar *et al.* (1996) observed that benzene hexachloride (BHC), organophosphorus (Malathion) and pyrethroid insecticides given orally at doses of 0.66 mg per rat, 0.06 mg per rat, 0.5 mg per rat respectively, to young adult

rats for 21 days resulted in significant reduction of serum concentrations of triiodothyronine (T_3), thyroxine (T_4) and T_4 / T_3 ratio. They also observed increased TSH level and no changes in body weight gain except pyrethroids toxicity.

Chronic or sub acute feeding of new generation pesticides such as acetochlor, clofentezine, mancozeb, pendimethalin, pentachloronitrobenzene and amitrole to rodents for 45 days increased thyroid hormone metabolism and excretion levels in the liver whereas TSH level was chronically elevated from pituitary for enhancing production of T_3 and T_4 by thyroid follicular cell hypertrophy and hyperplasia (Hurely *et al.*, 1998).

2.1.5.4 Reproductive Toxicity

2.1.5.4.1 Reproductive Toxicity of Fipronil

Hovda and Hooser (2002) reported that fipronil administration in female rats at a dose of 28.4 mg/kg /day in mating period resulted in reduction in litter sizes, body weight of litters, mating percentiles, post implantation survival and postnatal offspring survival.

Ohi *et al.* (2004) observed that topical application of fipronil at single dose of 70, 140 and 280 mg/kg body weight in rats caused alteration of endocrine function in the reproductive organs along with persistent diestrus mostly at dose of 280 mg/kg body weight. Litter size, birth weight, weaning weight and implantation were not affected by single dose of fipronil.

2.1.5.4.2 Reproductive Toxicity of Related Insecticides

Administration of endosulfan to rats at a dose as low as six milligram per kg per day from two weeks prior to mating through weaning resulted in a significant decrease in mean litter weight during lactation (Hoechst 1982). Maternal toxicity characterized by decreased body weight and increased relative liver weight was observed at six milligram per kg body weight per day.

Abdel-Khalik *et al.* (1993) investigated the effects of deltamethrin on fetuses in pregnant rats. They found early embryonic deaths were higher in deltamethrin treated groups.

Dalsender *et al.* (2003) observed no reduction in the body weight of the dams, litter size, number of viable young, body weight of offspring at birth and weaning, when female rats administered with endosulfan at dose levels of 0.5 or 1.5 mg/kg body weight.

2.1.5.5 Genotoxicity

2.1.5.5.1 Genotoxicity of Fipronil

Exposure of Chinese hamster lung cells in the presence of S9 fraction of rat liver to fipronil at 60 µg/ml produced chromosomal aberrations like chromatid breaks and chromatid exchanges (Fluoride Action Network, 2004).

2.1.5.5.2 Genotoxicity of Related Insecticide

Combination of cypermethrin and cadmium produced no chromosomal effects whereas cypermethrin-lead combinations induced a significant increase in the structural chromosomal aberrations by non sister chromatid exchange (Nehez *et al.*, 2000).

2.1.5.6 Hepatotoxicity of Fipronil

Hovda and Hooser (2002) reported that chronic feeding of fipronil in rats at a dose of 26.03 mg/kg/day for 90days resulted in decreased appetite, mean body weight and liver dysfunction. They also observed liver and thyroid were target organs for fipronil.

Exposure of human hepatocytes to doses of fipronil ranging from 0.1 to 25 µM resulted in cytotoxic effects due to apoptosis by induction of caspase-3/7 activity (Das *et al.*, 2006).

Fipronil was given orally in thyroid-intact female rats at the dose rate of three milligram per kilogram bodyweight for 14 days revealed decreased level of both total and free thyroid hormone and increased level of thyroid stimulating hormone. Fipronil induced a two fold increase of total and free thyroid hormone clearances by hepatic microsomal 4-nitrophenol UDP-glucuronosyltransferase enzyme (Julien *et al.*, 2009).

2.1.6 Biochemical and Haematological Effects

2.1.6.1 Biochemical and Haematological Effects of Fipronil

Fipronil sulfone metabolite administered in the diet of rats at doses of five, 50, 500 and 1000 ppm for 28 days revealed an increase of prothrombin time in males at 500 ppm and in females at 1000 ppm. The mean total cholesterol concentration was increased in females up to 35 per cent at 1000 ppm (Joint Meeting of FAO/WHO on Pesticidal Residues, 1997).

Fipronil-desulfinyl fed to rats at concentration of 0.5, two, and ten ppm for 104 weeks showed significant decrease in serum bilirubin, total protein and triglyceride concentrations in females at six and ten ppm whereas glucose and inorganic phosphate concentrations were increased at ten ppm (JMPR,1997).

Hovda and Hooser (2002) observed significantly higher bilirubin and creatinine levels whereas total protein, albumin, globulin and A/G ratio levels were significantly lower in the serum of rats when were administered with fipronil at doses of 26.03 mg/kg body weight and 28.4mg/kg for 90 days.

Rats were fed with 200 and 400 ppm of technical-grade of fipronil for 28 days showed marginal increment of platelets, decreased levels of total protein and globulin in all the animals. In females, cholesterol was increased at both doses whereas in males at 400ppm (Department for Environment Food and Rural Affairs, 2004).

Oral administration of technical-grade fipronil at 2.1mg/kg body weight to rats for 90 days revealed increased platelets counts along with decreased prothrombin time. ALT and AST were increased while serum proteins were decreased in both sexes at this dose level (Fluoride Action Network, 2004).

Administration of fipronil to beagle dogs at 10mg/kg body weight for 13 weeks revealed decreased serum cholesterol level at sixth and 12th week where as ALP and AST were increased up to 30 per cent at sixth week of administration (Anon., 2004).

As per FAN (2004) 18month dietary administration of fipronil in mice resulted in significant decreases in neutrophils up to 30 per cent and leukocytes numbers upto 28 per cent in females receiving 30 ppm on end of study.

Rats receiving fipronil at dose of 30 ppm in males and 300 ppm in females for 28 days revealed increased TSH level whereas T_4 levels were significantly decreased in all males at 300 ppm and females at 30 ppm (Department for Environment Food and Rural Affairs, 2004).

As per FAN (2004) dietary administration of fipronil to rats at dose 0.5, 1.5, 30 and 300 ppm for two years resulted in decreased serum albumin, total protein and albumin:globulin (A:G) ratio whereas bilirubin level was increased at 24, 50, 76 and 90th weeks. Platelets count and cholesterol levels were increased in males at 30 and 300 ppm.

Ohi *et al.* (2004) observed that topical application of single dose of fipronil at a dose of 70 mg/kg body weight to rats revealed increased progesterone level (64.0 ±13.4 nanogram/ml) when compared to control (29.0 ±3.6 nanogram/ml) 96 hours after dosing. Estradiol levels was reduced 96 hours after treatment with 70 mg/kg body weight of fipronil compared to control animals.

Wang *et al.* (2008) observed significant increase in AST and ALT levels in the serum of male rats following dietary administration of fipronil at doses of 10 and 25 mg/kg body weight for 90 days.

Vidau *et al.* (2009) observed the 200 µM of fipronil and its metabolites produced higher amount of lactate dehydrogenase from *in vitro* intestinal epithelium after 72 hours of exposure. However, the sulfone metabolite was more potent for inducing membrane damage than the fipronil and its sulfide metabolite.

2.1.6.2 Biochemical and Haematological Effects of Related Insecticide

Balasubramaniam *et al.* (1998) observed that oral administration of lindane at a dose of 20 mg/kg/day in rats for 30 days resulted increased levels of serum enzymes namely aspartate transaminase, alanine transaminase, alkaline phosphatase and the levels of thiobarbituric acid reactive substances, cholesterol, triglycerides and LDL-cholesterol.

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 (El- Tawil and Abdel-Rahman, 2001).

Omurtag *et al.* (2008) reported that wistar albino rats were administered endosulfan at a dose of 22 mg/kg/day, orally for five days revealed significant increase of proinflammatory mediators (TNF-alpha and IL-beta), LDH activity, AST, ALT, creatinine, cholesterol and BUN levels.

Remya (2008) observed that oral administration of cypermethrin at the dose levels of 80mg/kg and 120 mg/kg of body weight for 21 days in rats resulted reduction of packed cell volume, haemoglobin concentration, total erythrocyte count and total leukocyte count.

2.1.7 Antioxidant Assay of Related Compounds

Higher amount of superoxide radicals and lipid peroxides were observed in the liver of rat intoxicated with lindane whereas superoxide dismutase, catalase, reduced glutathione and GSH/GSSG ratio were decreased in the liver of rats (Videla *et al.*, 1988).

Barros *et al.* (1991) reported that rats treated with diets containing 20 ppm of α- or γ-hexachlorocyclohexane (HCH) for 15 or 30 days revealed increased production of lipid peroxides and superoxide anion in the liver by activity of cytochorome P-450 enzymes. Superoxide dismutase (SOD) activity was also increased in the liver of affected rats.

In liver, heart and skeletal muscles of hypothyroid rats (oxidative stressed), the lipid peroxidation was not modified, whereas in hyperthyroid rats, lipid peroxidation increased in liver and heart but not in skeletal muscles. The glutathione peroxidase activity increased significantly in the heart and skeletal muscle of both hypothyroid and hyperthyroid rats (Venditti *et al.*, 1997).

Ahamed *et al.* (2000) observed the thiobarbituric acid reacting substances, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and GGT were increased in the liver while GSH level was decreased in malathion poisong of rats at 20 ppm for four weeks.

Gabbianelli *et al.* (2002) reported that 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.

Deltamethrin increased malondialdehyde level and decreased activities of reduced glutathione, catalase, superoxide dismutase and glycogen in liver of rats (Manna *et al.*, 2004).

Thampan (2007) studied the pathology of deltamethrin toxicity in chick embryos and found that treatment at 200 and 400 ppm dose levels, it showed potent hepatotoxic effects mediated through free radicals as indicated by an increase in the lipid peroxidation and decrease in reduced glutathione and superoxide dismutase.

2.1.8 Gross Pathological Observations

2.1.8.1 Gross Pathological Observations on Fipronil

Dietary administration of fipronil destrifluoromethyl sulfonate for 28 days in rats at a dose of 10,000 ppm resulted in increased absolute and relative weight of liver, epididymides and testes. Prominent liver lobulation was noticed in females (Joint Meeting of FAO/WHO on Pesticidal Residues, 1997).

Oral administration of fipronil in pregnant rats at 2.5 mg/kg body weight for seven days revealed decreased ossification of fetal bones such as hyoid, $5^{th}/6^{th}$ sternebrae, 1^{st} thoracic vertebral body, pubic bone and metatarsi (United States Environmental Protection Agency, 1998).

Tingle *et al.* (2003) found that oral administration of fipronil desulfinyl in dogs at the dose of 27 ppm for 28 days resulted in reduction in thymus weights with pale livers.

Fipronil was given orally at one, five, 30 and 300 ppm for 13 weeks resulted in significant increase of absolute liver weight in males at 300 ppm while in the females at doses of one to 300 ppm. Absolute thyroid weights were elevated at dose 30 ppm in females and at 300 ppm in males (Department for Environment Food and Rural Affairs, 2004).

Dietary administration of fipronil to rats for two years resulted in increased liver weights in both the sexes at 300 ppm. Thymus and uterus weights were decreased in females at 300 ppm. Pale and enlarged kidney was noticed in both the sexes at 30 ppm. Enlargement of livers, thyroid and adrenals were observed in males at 300 ppm (Fluoride Action Network, 2004).

Anon (2004) observed whitish necrotic foci in the liver of mice receiving 30 ppm fipronil for 18 months.

In a two-generation study, rats administered with fipronil in the diet at dose of three, 30 and 300 ppm at mating period in males whereas in females at

gestation and lactation period revealed increased weight of liver and thyroid at dose of 30 and 300 ppm in both the sexes. Pituitary gland weight was decreased in females at a dose of 300ppm (Department for Environment Food and Rural Affairs, 2004).

Wang *et al.* (2008) found the necrotic foci in the liver of rats in sub chronic toxicity of fipronil. The dose employed were 10 and 25 mg/kg body weight for 90 days.

2.1.8.2 Gross Pathological Observations of Related Insecticide

Yaqoob *et al.* (1995) reported that adult female rabbits fed 1.5 mg/kg of endosulfan for 12 weeks showed enlarged fragile liver with congestion and necrotic changes.

Junqueira *et al.* (1997) observed that single administration of lindane at a dose of 60 mg/kg body weight produced severe congestion, haemorrhage and necrotic foci in the liver.

In a study of the combined effect of cypermethrin, arsenic and mercury, significant changes in the weights of liver, kidney and adrenal were reported by Institoris *et al.* (1999).

Wade *et al.* (2002) reported that Sprague-dawely rats administered with endosulfan at one micro liter per kilogram bodyweight by gavage for 70 days resulted in significant increase of liver and kidney weights.

2.1.9 Histopathological Observations

2.1.9.1 Histopathological Observations of Fipronil

Fipronil-desulfinyl fed to rats at concentration of 0.5, two, and ten ppm for 104 weeks resulted increased incidence of pituitary (pars distalis) adenomas in males and mild diffused hepatocyte hypertrophy in females at a dose of 10 ppm (Joint Meeting of FAO/WHO on Pesticidal Residues, 1997).

Increased sinusoidal lymphoid aggregations, degenerative changes concomitant with fine vacuolation of hepatocytes at periportal region and follicular epithelial hypertrophy in the thyroid gland were observed in rats at 10,000 ppm of fipronil destrifluoromethyl sulfonate for 28 days (Joint Meeting of FAO/WHO on Pesticidal Residues, 1997).

Tingle *et al.* (2003) observed arteritis and myocardial necrosis in dogs administrerd with 35 ppm fipronil-desulfinyl for 28 days.

Progressive senile nephropathy in male rats and thyroid follicular cysts in female rats were the lesions observed when fipronil was administered at dose of 30ppm for two years. After 52 weeks, incidence of thyroid follicular cell adenomas and carcinomas were increased in both the sexes at 300 ppm (Anon., 2004).

Dietary administration of fipronil (95.4 per cent purity) at dose of 30 and 300 ppm in rats for 13-weeks revealed increased thyroid follicular cellular hyperplasia and hypertrophy along with congestion of liver and thyroid. Pan-acinar hepatic fatty vacuolation was observed in males only at a dose of 300ppm (Fluoride Action Network, 2004).

Thyroid follicular-cell hypertrophy and generalized enlargement of hepatocytes were the lesions observed in rats administrated with technical-grade fipronil at a dose of 200 and 400 ppm for 28 days (Department for Environment Food and Rural Affairs, 2004).

Significant increase of micro vascular periacinar vacuolation in the liver was reported among mice dosed with fipronil at the dose of 10ppm for 18 months (DEFRA, 2004).

As per WHO (2007) in a twenty eight days study of toxicity in mice, fipronil-desulfinyl increased the incidence of centrilobular hypertrophy of the hepatocytes in both the sexes at a dose of 30 ppm.

The histopathological effect of fipronil in rats at a dose of 25mg/kg for 90days was demonstrated by wang *et al.* (2008). They observed cloudy swelling of hepatocytes at around the central vein of the liver.

2.1.9.2 Histopathological Observations of Related Insecticide

Local inflammation, hemorrhage and dilated alveoli of the lungs were noticed in rats administered with endosulfan at a dose of 10mg/kg body weight/day for 15 days (Gupta and Chandra, 1977).

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

Wade *et al.* (2002) observed that sub chronic exposure of 16 organchlorines, lead and cadmium mixture at the dose level of one ppm, 10ppm, 100 ppm and 1000 ppm in male sprague-dawely rats for 70 days revealed decreased amount of colloid within follicles and increased size and vacuolization of follicular epithelium in thyroid gland whereas hypertrophy and fatty change were the lesions in the liver observed at a dose of 1000ppm.

Rats fed with single oral dose of deltamethrin at 150mg/kg (LD50) revealed marked congestion in the brain, spleen, heart and testes. Lungs revealed severe haemorrhage whereas liver showed severe necrosis (Manna *et al.*, 2004).

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).

Remya (2008) observed fusion of villi, diffuse hyperplasia of the goblet cells with focal infiltration of inflammatory cells into the lamina propria and sub mucosa of the intestine of rats receiving cypermethrin at a dose of 80 mg/kg body weight for 21days.

2.2 CURCUMIN

Recently, interest in complementary and alternative medicine has grown rapidly in industrialized countries and the demand for herbal remedies has currently increased. *Curcuma longa* {[1, 7-bis (4-hydroxy 3-methoxy phenyl)-1, 6-heptadiene-3,5-dione]}commonly known as turmeric belongs to the zingiberaceae or ginger family. The most active component in turmeric is curcumin (diferuloylmethane) derived by ethanolic extraction (Ammon and Wahl, 1991).

2.2.1. Pharmacokinetics

Wahlstrom and blennow (1978) reported that when curcumin administered at the dose of one gram per kilogram of body weight to Sprague-Dawely rats 75 per cent was excreted in faeces, while negligible amount appeared in urine.

Shoba *et al.* (1998) observed that curcumin had poor bioavailability due to its rapid metabolism in the liver and intestinal wall. When curcumin was given alone, in the dose of two gram per kilogram of body weight to rats, moderate serum concentrations were achieved over a period from four hours. Piperine (20 mg/kg body weight) increased absorption, serum concentration and bioavailability of curcumin in both rats and humans by inhibition of hepatic and intestinal glucuronidation of curcumin.

Lin *et al.* (2000) found that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and that these compounds subsequently were converted to monoglucuronide conjugates in mice.

2.2.2 Mechanism of action

Curcuma longa has traditionally been used for treatment of gastrointestinal colic, flatulence, hemorrhage, hematuria, menstrual difficulties and jaundice. Curcumin has good pharmacological properties including antioxidant, anti-inflammatory, thrombolytic, antimutagenic, hepatoprotective, antiparasitic and anticancer activity (Miquel *et al.*, 2002).

2.2.3. Ameliorative Effect of Curcumin

Soudamini *et al.* (1992) investigated the effect of oral administration of curcumin on serum cholesterol levels and on lipid peroxidation in the liver, lung, kidney and brain of mice treated with carbon tetrachloride, paraquat, and cyclophosphamide. The results indicated that curcumin had lowered the increased peroxidation of lipids in these tissues and lowered the serum and tissue cholesterol levels in mice.

An investigation on the effect of one percentage turmeric in the diet of chicken affected with *Eimeria maxima* for three weeks of age showed that turmeric had a protective effect on weight gain in *E.maxima* affected chicken and reduced lesion spores and oocysts output (Allen *et al.*, 1998).

Venkatesan (1998) reported that curcumin when administered orally at dose level of 200 mg/kg body weight for seven days before and two days following administration of adriamycin (ADR) in rats caused significant amelioration of cardiotoxicity such as ST segment elevation and increased heart rate. Curcumin also prevented the rise of serum CK and LDH enzymes exerted by ADR. ADR-treated rats administered with curcumin displayed a significant inhibition of lipid peroxidation and limited the free-radical-mediated organ injury.

Venkatesan (2000) studied the pulmonary protective effect of curcumin against paraquat (PQ) toxicity in rats. Curcumin at a dose level of 300mg/kg prevented the general toxicity and mortality of rats. Curcumin also blocked rise of

bronchoalveolar lavage fluid (BALF) protein, angiotensin-converting enzyme (ACE), alkaline phosphatase (ALP), N-Acetyl-beta-D-glucosaminidase (NAG), thiobarbituric acid reactive substances (TBARS) and neutrophils.

Venkatesan *et al.* (2000) found that oral administration of curcumin (200 mg/kg body weight) markedly protected against adriamycin induced proteinuria, albuminuria and hypoalbuminemia whereas plasma cholesterol was decreased by curcumin. Curcumin also protected against adriamycin induced renal injury by suppressing oxidative stress and increasing kidney glutathione content and glutathione peroxidase activity.

Shukla *et al.* (2003) observed that concurrent treatment of curcumin (100mg /kg orally) and lead (50mg/kg orally) for 45days in rats caused a significant decrease in lead levels in all the brain regions as compared with lead alone. They also observed a significant increase in reduced glutathione and superoxide dismutase levels in all the brain regions in rats simultaneously treated with lead and curcumin.

Padmaja and Raju (2005) found that selenium administration resulted in a marked decrease in the activity levels of the liver succinate dehydrogenase, malate dehydrogenase and lactate dehydrogenase while pyruvate dehydrogenase increased significantly in the wistar rats. Curcumin prevented oxidative damage mediated by selenium and protective effect of dehydrogenases by its anti-oxidative property.

Mathuria and Verma (2007) found that oral administration of aflatoxin (750 and 1500 mg/kg body weight) along with curcumin (50 mg/kg body weight) resulted in a dose-dependent significant reduction of DNA, RNA and protein contents in the liver and kidney of mice, as compared to aflatoxin alone treated groups.

Acute hepatotoxicity was induced in rats by oral administration of CCl_4 at four gram per kilogram body weight. Curcumin was given at dose level of 200 mg/ kg body weight by orally before and two hours after CCl_4 administration. This prevented development of necrosis and cholestasis in the liver due to CCl_4 intoxication (Gordillo *et al.*, 2007).

Yadav *et al.* (2009) observed that oral administration of curcumin simultaneously with arsenic at the dose level of 100 mg/kg body weight and 20 mg/kg body weight respectively for 28 days revealed increased locomotor activity and grip strength

2.2.4. Effects on Haematological and Biochemical Parameters

Rao *et al.* (1970) found that dietary curcumin (0.1 per cent or 0.5 per cent) increased faecal excretion of bile acids and cholesterol both in normal and hypercholesteremic albino rats. Curcumin restored body and liver weights by correcting the ill effects of high cholesterol diet

Deshpande *et al.* (1998) observed that concurrent treatment of turmeric extract and carbon tetrachloride reduced the serum level of bilirubin, cholesterol, aspartate aminotransferase (AST) and alkaline phosphatase in experimentally induced hepatotoxicity in rats.

Park *et al.* (2000) investigated the effect of curcumin on acute and subacute carbon tetrachloride toxicity and observed that curcumin at doses of 100 mg/kg and 200mg/kg ameliorated toxicity. Curcumin lowered the activity of ALT and AST in the carbon tetrachloride induced liver compared to the control group.

Al–Sultan (2003) found that higher levels of turmeric inclusion (0.5 and 1 percentage) in diets increased both total erythrocyte and leukocyte count of broiler chicken.

Concomitant dietary administration of curcumin and endotoxins in rats respectively at doses of 60mg/kg and one mg per kg for seven days significantly reduced the elevated levels of AST, ALT, ALP and bilirubin compared to endotoxins treated group (Kaur *et al.*, 2006).

Serum γ-glutamyl transpeptidase (γ-GTP), alanine aminotransferase (ALT) and total bilirubin levels were significantly decreased in rats treated with curcumin and CCl_4 compared to rats treated with CCl_4 alone (Gordillo *et al.*, 2007).

Simi (2007) reported the effect of dietary turmeric on production performance in broilers and observed that the mean values of hemoglobin, TLC and total protein in the turmeric supplemented group were significantly higher than that of control group. Decreased levels of liver enzymes like ALT and AST in the turmeric supplemented group were also noticed.

Mathuria and Verma (2008) observed that oral administration of curcumin along with aflatoxin to mice caused significant reduction of serum parameters such as creatinine, total protein, aspartate aminotransferase and alanine aminotransferase levels compared to the aflatoxin alone treated groups.

2.2.3. Antioxidant Activity

Ammon and Wahl (1991) opined that curcumin protected oxidative degradation of lipids and haemoglobin in rats. DNA protective activity of curcumin was higher than other well known biological antioxidant like lipoate, alpha-tocopherol and beta-carotene.

Oetari *et al.* (1996) reported that curcumin was a potent inhibitor of glutathione S-transferase (GST) in cytosol of rat liver by its antioxidant properties.

Jayaprakasha *et al.* (2006) reported higher antioxidant activity in curcumin followed by demethoxycurcumin and bisdemethoxycurcumin at 100ppm compared to butylated hydroxyl toluene (BHT) at 100ppm by *in vitro* model systems, such as the phosphomolybdenum and linoleic acid peroxidation methods.

Gordillo *et al.* (2007) found a significant reduction of glutathione, GSH/GSSG ratio and total liver glutathione levels in rats treated with CCL_4. Oral administration of curcumin at the rate of 200mg/ kg in the CCL_4 treated group showed significant increase in all the above values which indicated the protective effect. However, curcumin was not capable to decrease lipid peroxides levels in the acute CCL_4 toxicity.

Dietary administration of curcumin at 50mg/kg body weight along with aflatoxin at 1500mg/kg body weight to mice for 45 days resulted in significant increase of catalase, superoxide dismutase, glutathione peroxidase and glutathione in the liver and kidney (Verma and Mathuria, 2008).

Yadav *et al.* (2009) studied simultaneous treatment of curcumin and arsenic in rats at 20mg/kg body weight and 100mg/kg body weight respectively for 28 days and found decreased lipid peroxides levels and increase in reduced glutathione content in the brain compared to rats treated with arsenic alone.

Cekmen *et al.* (2009) noticed that simultaneous treatment of curcumin at 200mg/kg body weight and acetaminophen at dose level of 1000mg/kg body weight(intra peritoneally)in rats resulted into higher production of glutathione peroxides, catalase and superoxide dismutase along with decreased lipid peroxide level in the kidney compared to rats treated with acetaminophen alone.

2.2.4. Histopathological Examination

Glomerulosclerosis, tubular degeneration and cellular infiltration in the kidney were decreased when curcumin was fed at 0.5 percentage to diabetic rats for eight weeks (Babu and Srinivasan, 1998).

Kaur *et al.* (2006) reported that seven day curcumin diet reduced kupffer cell hyperplasia, neutrophils infiltration and necrotic foci in the liver when single dose of endotoxins was given intraperitoneally at one milligram per kilogram body weight.

Infiltration of the intertubular connective tissue was very much reduced in the kidney following oral administration of curcumin at dose level 200mg/kg body weight/day simultaneously with gentamicin at 100mg/ body weight/day for 21 days (Farombi and Ekor, 2006).

CCl_4 produced severe necrotic foci and distortion of liver around portal triads in rats which were found to be greatly reduced when treated with curcumin (Gordillo *et al.*, 2007).

Kumar *et al.* (2008) reported that pre-treatment of mice with curcumin at dose level of 50 mg/kg body weight for 14days resulted in hepatoprotection against paracetamol induced injury.

Cekmen *et al.* (2009) reported that rats treated with single dose of acetaminophen and oral curcumin at dose level of 1000mg/kg and 200mg/kg intra peritoneally decreased tubular degeneration and epithelial vacuolization in the proximal convoluted tubules compared to acetaminophen treated group.

In curcumin-pretreated rats, the integrity of the hepatocytes were relatively well preserved compared with massive and severe hepatocyte necrosis at the centrilobular zone in dimethylnitrosamine induced liver injury (Farombi *et al.*, 2008)

Materials and Methods

3. MATERIALS AND METHODS

3.1 INSECTICIDE

Technical grade fipronil was procured from Gsp Crop Science Pvt. Ltd., Gujarat.

3.2 EXPERIMENTAL ANIMALS

Adult male wistar albino rats weighing approximately 150-200 grams procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for this study. Rats were maintained on identical feeding and management practices in the laboratory for two weeks before the commencement of studies. The experiment was conducted for a period of 28 days.

3.3 INSECTICIDE SOLUTION

Technical grade fipronil (98.06 per cent) was made into a suspension in 1.5ml of honey and the required dose was administered orally

3.4 EXPERIMENTAL DESIGN

3.4.1 Fixation of Dose Level

Rats were administered with different dose levels of fipronil (10.5, 21, 30 and 45mg/kg body weight/day) and kept for 28days. Those showing visible histopatho logical changes in the organs were studied and the dose was fixed accordingly. The final dose of study was 30.4 mg/kg body wt/day.

The experiment was conducted as per schedule shown in the table.

Group	Treatment
Group -1	Fipronil (30.4 mg/kg body wt/day) was made into a suspension in honey at 1.5ml/rat.Then mixed suspension was administered orally for 28 days.
Group -2	Fipronil (30.4mg/kg body wt/day) and curcumin (100 mg/kg) were made into a suspension in honey at 1.5ml/rat Then mixed suspension was administered orally for 28 days..
Group -3	Healthy control was administered with honey at the rate of 1.5 ml/rat/day orally for 28 days.

3.5 PARAMETERS

3.5.1 Body Weight

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

3.5.2 Haematological Parameters

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

Total Leukocyte count (TLC), Packed Cell Volume (PCV) and Differential Leucocyte count (DLC) were estimated by the method suggested by Thrall *et al.* (2004) on days 0, 7th, 14th, 21st and 28th. Concentration of Haemoglobin (Hb) was estimated by acid haematin method as described by Feldman *et al.* (2000).

Erythrocyte Sedimentation Rate (ESR) was estimated by wintrobe haemotocrit tube method (Benjamin, 1985).

3.5.3 Biochemical Studies

Blood was collected from the retro-orbital plexus under mild diethylether anaesthesia with capillary tubes into clean vials (heparinised) and allowed to clot. The serum was separated from the clot and then it was centrifuged at 2000 rpm for 20 minutes. The clear serum was aspirated into another vial and used for biochemical analysis. Total serum protein was estimated by Biuret method (Henry *et al.*, 1957). Albumin was estimated by Douma's method (Doumas *et al.*, 1971) using kit supplied by Agappe Diagnostics Pvt. Ltd, Ernakulam, Kerala. Serum creatinine was estimated by modified Jaffe's method using commercial kits purchased from Agappe Diagnostics. Estimation of serum AST and ALT was done using kits manufactured by Agappe diagnostics (Reitman and Frankel, 1957). Cholesteol estimation was carried out according to the simplified method (Abell *et al.*, 1952) using kit supplied by Agappe Diagnostics.

3.5.4 Reduced Glutathione, Lipid Peroxides and Superoxide Dismutase

The animals were sacrificed and dissected upon and the liver was collected. It was washed in running tap water to remove the blood clots and weighed amount of tissue was kept in chilled normal saline.

3.5.4.1 Estimation of Tissue Reduced Glutathione

Levels of reduced Glutathione in liver homogenate were estimated by the method of Moron *et al.* (1979).

a. Principle

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

b. Reagents

Disodium hydrogen phosphate, monosodium dihydrogen phosphate and DTNB were purchased from Himedia Laboratories Pvt. Ltd., Mumbai. Trichloroacetic acid was procured from Qualigens Fine Chemicals, Glaxo Smith Kline Pharamaceuticals Ltd., Mumbai. Mono and disodium hydrogen phosphates were used for the preparation of phosphate buffer.

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

c. Procedure

1. Preparation of tissue homogenate:

Homogenate of liver was prepared in the ratio of 0.5 g of wet tissue in four ml of phosphate buffer. It was then centrifuged at 5000 rpm and the supernatant was used for the estimation of reduced glutathione.

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

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

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

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

To the above mixture, two ml of freshly prepared DTNB was added, mixed well and the yellow colour formed was read at 412 nm.

d. Preparation of standard curve:

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

3.5.4.2 Estimation of Lipid Peroxides

Level of lipid peroxides in tissue homogenate was determined by the method of Ohkawa *et al.* (1979).

a. Principle:

Thiobarbituric acid reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that can be determined by colorimetry. TMP was used as a

standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

b. Reagents:

TBA and TMP were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Sodium dodecyl sulphate (SDS) was procured from Sigma-Aldrich India, Bangalore: and all the other chemicals were purchased from Merck India Limited, Mumbai.

1. 8.1 per cent SDS
2. 20 per cent acetic acid solution, pH adjusted to 3.5 with NaoH
3. 0.8 per cent aqueous soluation of TBA
4. 1.15 per cent KCI

c. Procedure:

Preparation of tissue homogenate:

Homogenates of liver was prepared in a ratio of one gram of wet tissue to nine ml of 1.15 per cent KCL solution (10 per cent w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for five minutes and the supernatant was used for the estimation of lipid peroxides.

To 100 micro liter of the supernatant, added 200 µl of 8.1 per cent SDS, 1.5ml 20 per cent acetic acid solution (pH 3.5) and 1.5ml of 0.8 per cent aqueous solution of TBA. The mixture was made up to 4 ml with distilled water, and heated in a water bath at 95 °C for 60 minutes. After cooling under tap water, one ml of distilled water and five ml of n-butanol

were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, absorbance of the organic layer was taken at 532 nm.

d. Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 nM to 5 nM of TMP in deionised double distilled water by following the above procedure. A graph was plotted between optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nmol of malondialdehyde/g wet tissue.

*3.5.4.3 **Estimation of Superoxide dismutase***

Superoxide Dismutase was estimated according to the procedure followed by Mimami and yoshikawa (1979).

a. Reagents

1. Tris cacodylic acid buffer (50mM, pH 8.2)

   Tris cacodylic acid 50mM

   Dietheylene triamine penta acetic acid 1mM

   Nitroblue tetrazolium 0.1mM

   Triton X 100 0.001 percent

   All the reagents were mixed in equal quantities and the pH was adjusted to 8.2 using 0.1N sodium hydroxide.

2. Sodium chloride 0.9 percent

3. Pyrogallol 0.2mM

b. Procedure

1. 200mg of freshly excised liver was homogenized with 2.5 ml of 0.9 percent sodium chloride followed by centrifugation at 400 rpm for 10 minutes at 4°C to harvest the supernatant

2. The assay mixure in a total volume of 3ml consisted of 1.4ml of 50mM tris cacodylic acid, 1.4ml of 0.2mM Pyrogallol and 0.2ml of enzyme preparation.

3. Blank contained distilled water instead of enzyme preparation.

4. The absorbance due to autooxidation of pyrogallol was read at 420nm using geneys spectrophotometer.

5. One unit of SOD activity was the amount of enzyme which inhibited pyrogallol autooxidation by 50 per cent under experimental conditions.

6. The values were expressed in units /mg of protein after quantifying the protein content of supernatant by method of Lowry *et al.* (1951).

3.6 PATHO ANATOMICAL STUDIES

At the end of the experiment, animals were sacrificed. Detailed postmortem examination was conducted and gross lesions were noted. Liver, kidney, brain, thyroid, spleen, heart, stomach and intestine were collected for histopathology. Tissues were fixed in 10 per cent formalin. The tissues were processed and paraffin embedded as described by Sheehan and Hrapchak (1980).

Sections were cut at 4 micron thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Gamble, 2008).

3.7 STATISTICAL ANALYSIS

Data collected from various parameters were analyzed as per the method of Snedecor 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 the study of fipronil toxicity and its ameliorative effect with curcumin in rats are tabulated and presented in tables and graphs/ diagrams.

4.1 PHYSIOLOGICAL PARAMETERS

4.1.1 Body Weight

The individual and mean body weight of rats of group I, II and III were recorded on 0, 7^{th} 14^{th}, 21^{st} and 28^{th} day of the experiment and are presented in the table 1 and figure1. Body weight of rats of all groups showed a gradual increase throughout the experimental period. On day 7^{th},14^{th},21^{st} and 28^{th} fipronil group(group I) showed significantly($p<0.05$) lower mean body weights(193.36 ±1.18g, 201.11 ±1.67g, 199.60 ±0.72g and 211.26 ±2.18g respectively)compared to fipronil and curcumin group(group II)mean body weights (198.81 ±1.81g, 207.42 ±1.17g, 214.24 ±1.03g and 220.13 ±1.73g respectively)and control(group III)mean body weights(205.04 ±1.17g,213.64 ±0.95g, 221.23 ±1.38g and 232.31 ±1.88g respectively).Group II showed significantly($p<0.05$) lower mean body weights compared to group III whereas significantly($p<0.05$) higher mean body weight was observed compared to group I .

4.2. BIOCHEMICAL PARAMETERS

4.2.1 Total Protein

The mean total protein levels of group I, II and III at weekly intervals are shown in table 2 and figure 2. On day 7^{th} ,14^{th} and 21^{st} group I showed significantly ($p<0.05$) lower protein levels(6.01 ±0.10 g/dl, 5.61 ±0.06 g/dl and 5.18 ±0.08 g/dl respectively)compared to group II levels(6.36 ±0.10g/dl,6.24 ±0.14g/dl and 6.17 ±0.10g/dl respectively) and group III levels(6.37 ±0.05 g/dl, 6.36 ±0.12g/dl and 6.41 ±0.11g/dl respectively).No significant difference was noticed between group II and group III on days 7^{th} 14^{th} and 21^{st}. On day 28^{th}, group I showed significantly ($p<0.05$) lower mean protein level (4.91 ±0.08g/dl) compared to group II (6.04 ±0.12g/dl) and group III

(6.47 ±0.11g/dl). Group II showed significantly ($p<0.05$) lower mean protein level compared to group III but significantly higher level than group I.

4.2.2 Albumin

The mean albumin levels of group I, II and III at weekly intervals are shown in table 3 and figure 3. On day 14th and 21st, group I showed significant lower albumin levels (3.00 ±0.12g/dl and 2.86 ±0.08 g/dl respectively) compared to group II albumin level (3.46 ±0.07 g/dl and 3.42 ±0.13 g/dl respectively) and group III (3.58 ±0.10 g/dl and 3.62 ±0.04 g/dl respectively).No significant difference was observed between group II and group III. On day 28th, group I showed significantly ($p<0.05$) lower mean albumin level (2.74 ±0.10g/dl) compared to group II albumin level (3.25 ±0.11g/dl) and group III level (3.64 ±0.08g/dl) meanwhile group II showed significantly($p<0.05$) higher albumin level compared to group I and significantly($p<0.05$) lower albumin level compared to group III. On day 7th, no significant difference was observed between all the three groups.

4.2.3 Globulin

The mean globulin levels of group I, II and III at weekly intervals are shown in table 4 and figure 4. On 21st and 28th days, group I showed significantly ($p<0.05$) lower globulin levels (2.32 ±0.09 g/dl and 2.17 ±0.12 g/dl respectively) compared to group II levels (2.75 ±0.10g/dl and 2.79 ±0.15g/dl respectively) and group III levels (2.79 ±0.08 g/dl and 2.83 ±0.14g/dl respectively). No significant difference was noticed between group II and group III. On days 7th and 14th, no significant difference was noticed between all the three groups.

4.2.4. Serum Aspartate Aminotransferase (AST)

The mean aspartate aminotransferase(AST) level of group I, II and III at weekly intervals are shown in table 5 and figure 5. On day 7th, group I showed significantly ($p<0.05$) higher AST level (201.68 ±3.46U/L) compared to group II level (192.96 ±3.65U/L) and group III level (181.26±1.44U/L). Group II showed significantly ($p<0.05$) lower AST level (192.96 ±3.65U/L) compared group I level on day 7. No significant difference was observed between group II and group III. On day 14th, group I showed

significantly ($p<0.05$) higher AST level (226.26 ±2.5U/L) compared to group III level (183.16 ±2.34U/L).Group II showed significantly ($p<0.05$) higher AST level (189.3 ±2.86U/L) compared to group III level (183.16 ±2.34U/L) whereas no significance difference was observed between group I and group II. On day 21st and 28th, group I showed significantly ($p<0.05$) higher AST levels (229.88 ±1.63 U/L and 258.24 ±1.32 U/L respectively) compared to group II levels (193.28 ±1.49U/L and 199.82 ±1.23U/L respectively) and group III levels (182.68 ±1.34U/L and 181.56 ±1.09 U/L respectively) whereas group II showed significantly ($p<0.05$) higher AST levels compared to group III but significantly ($p<0.05$) lower AST level compared to group I.

4.2.5. Serum Alanine Aminotransferase (ALT)

The mean alanine aminotransferase(ALT) levels of group I, II and III at weekly intervals are shown in table 6 and figure 6.On day 7th ,14th ,21st and 28th, group I showed significantly($p<0.05$) higher mean ALT levels (86.78 ±0.65 U/L,93.28 ±0.76 U/L,98.6 ±2.41 U/L and 102.26 ±3.4 U/L respectively) compared to group II levels (69.16 ±0.94 U/L ,72.48 ±0.90 U/L,75.74 ±2.43 U/L and 78.26 ±0.97 U/L respectively) and group III levels (63.72 ±0.58 U/L, 62.86 ±0.68 U/L, 65.16 ±1.18 U/L and 63.16 ±0.83 U/L respectively). On day 7th, 14th, 21st and 28th, group II showed significantly ($p<0.05$) lower ALT levels compared to group I but significantly ($p<0.05$) higher ALT levels compared to group III.

4.2.6 Serum Cholesterol

The mean cholesterol levels of group I, II and III at weekly intervals are shown in table 7 and figure 7. On 21st and 28th days, group I showed significantly ($p<0.05$) higher cholesterol levels (51.18 ±0.57mg/dl and 52.34 ±0.54mg/dl respectively) compared to group II levels (47.68 ±0.58mg/dl and 47.5 ±0.79mg/dl respectively) and group III (49.75 ±0.47mg/dl and 50.25 ±0.76mg/dl respectively).Group II showed lower cholesterol levels throughout the experimental period compared to group III. There was no significant difference between group II and group III. On 21st and 28th days, group II showed significantly ($p<0.05$) lower cholesterol levels (47.68 ±0.58 mg/dl and 47.5 ±0.79 mg/dl respectively) compared to group I levels (51.18 ±0.57 mg/dl and 52.34 ±0.54

mg/dl respectively). No significant difference in the cholesterol levels was observed between the treatment groups on days 7th and 14th.

4.2.7 Creatinine

The mean levels of creatinine are presented in table 8. On day 28th, group I showed significantly ($p<0.05$) higher mean creatinine level (0.85 ±0.04 mg/dl) compared to group II (0.64 ±0.05 mg/dl) and group III (0.63 ±0.02 mg/dl). Group II showed significantly ($p<0.05$) lower creatinine level compared to group I. No significant difference was observed between group II and group III. On day 7th, 14th and 21st, no significant difference in the creatinine levels was observed between the treatment groups.

4.3 HAEMATOLOGICAL PARAMETERS.

4.3.1 Packed Cell Volume (PCV)

The mean packed cell volume of group I, II and III at weekly intervals are shown in table 9 and figure 8. On days 14th, 21st and 28th, group I showed significantly ($p<0.05$) lower mean PCV values (43.30 ±0.68 per cent, 42.97 ±0.58 per cent and 42.11 ±0.53 per cent respectively) compared to group II (44.13 ±0.39 per cent, 44.19 ±0.43 per cent and 43.90 ±0.56 respectively) and group III values (45.29 ±0.47 per cent, 45.15 ±0.48 per cent and 45.37 ± 0.42 per cent respectively) whereas group II showed significantly ($p<0.05$) higher PCV values compared to group I values. No significant difference was noticed between group II and group III.

4.3.2 Haemoglobin (Hb)

The mean values of haemoglobin of group I, II and III at weekly intervals are shown in table 10. No significant difference was observed between all the three groups at days 0, 7th, 14th and 21st. On day 28th, group I showed significantly ($p<0.05$) lower Hb value (14.21 ±0.35 g/dl) compared to group II value (15.02 ±0.39 g/dl) and group III value (15.43 ±0.36g/dl) whereas no significant difference was noticed between group II and group III on that day.

4.3.3 Erythrocyte Sedimentation Rate (ESR)

The average erythrocyte sedimentation rate of group I, II and III at weekly intervals are shown in table 11. No significant difference was observed between all the three groups.

4.3.4 Total Leukocyte Count (TLC)

The mean TLC values are listed in the table 12. Results indicated that there were no variations in the TLC values between the three groups.

4.3.5 Differential Leukocyte Count (DLC)

The mean differential leukocyte count of group I, II and III at weekly intervals are shown in table 13 and 14. No significant difference was observed between all the three groups.

4.4 MORTALITY PATTERN

4.4.1 Group I (fipronil alone)

One rat died on day three with convulsion and excitability. One more death occurred on day four with no specific signs. Remaining eight rats survived till the end of experiment.

4.4.2 Group II (fipronil and curcumin group)

One rat died on the 3rd day due to biting injury by other animals. Remaining nine rats survived till the end of the experiment.

4.4.3. Group III (control)

No mortality was observed in this group. The rats remained active during the whole 28 days study period.

4.5 CLINICAL SIGNS

4.5.1. Group I

Abnormal gait, convulsions and aggressiveness were observed in rats which died on day three. The rat which died on day four exhibited dullness and depression (Fig 12). The survived rats showed reduced feed intake throughout the experimental period. The weight gain by the survived rats was also less compared to the group II and control group rats.

4.5.2 Group II

The rat which died on day three showed inappetence and depression. The survived rats showed reduced feed intake for seven days and then returned to normal feeding habits. The weight gain by the survived rats was also less compared to the control group of rats.

4.5.3 Group III

The rats in the control group did not show any symptoms till completion of the study. The feeding habit was quiet normal and all the animals remained healthy.

4.6 OXIDATIVE EFFECT ON LIVER

4.6.1 Lipid Peroxides

The mean levels of lipid peroxides in the liver of group I, II and III are presented in table 15 and figure 9.Group I showed significantly ($P<0.05$) higher mean lipid peroxides level (452.44 ±15.58nMol/g) compared to group II level (336.27 ±8.37nMol/g) and group III level (325.06 ±6.26nMol/g).Group II showed significantly lower lipid peroxide level compared to group I. No significant difference was observed between group II and group III.

4.6.2 Reduced Glutathione

The mean levels of reduced glutathione in group I, II and III are listed in table 15 and figure 10. Group I showed significantly ($P<0.05$) lower reduced glutathione level (435.35 ±6.34 µg/g) compared to group II level (513.85 ±4.49µg/g) and group III level (526.39 ±5.5µg/g). Group II showed significantly ($P<0.05$) higher level compared to group treated with fipronil alone. No significant difference was observed between group II and group III.

4.6.3 Superoxide Dismutase

The mean levels of superoxide dismutase of group I, II and III are listed in table 15 and figure 11. Group I showed significantly ($P<0.05$) lower mean superoxide dismutase levels (42.36 ±0.85 Units /mg of protein) when compared with group II (48.82 ±0.58 Units /mg of protein) and group III (50.24 ±0.90 Units/mg of protein).Group II showed significantly ($P<0.05$) higher mean level compared to group I. No significant difference was noticed between group II and control group III

4.7 PATHOANATOMICAL STUDIES

4.7.1 Gross lesions

4.7.1.1 Group I

Hepatomegaly and congestion were noticed in the liver of all the rats. Grayish white spots of necrosis were observed grossly in the liver of three rats (Fig 13). Four rats showed moderate congestion and haemorrhage in the lungs (Fig 14). In all the rats, thyroids were found to be enlarged and hard in consistency (Fig 15). Kidneys revealed congestion and focal haemorrhage. Two animals showed mild congestion in the brain. No gross changes were noticed in the spleen, heart, stomach and intestine.

4.7.1.2 Group II

Thyroid was slightly enlarged in three rats (Fig 16). Moderate congestion was noticed in the liver of all the animals. No gross changes were noticed in the kidney, stomach, lung, intestine and spleen of all the animals. The rats which died during the course of experiment showed no gross lesions in the organs examined.

4.7.1.3 Group III

The rats maintained with administration of honey were sacrificed on 28th day of experiment No apparent gross lesions were observed in any of the organs during postmortem.

4.7.2 Histopathology

Histopathological examination of various organs of rats, which died during the course of the experiment, did not reveal any changes. Sacrificed animals showed microscopical changes after completion of the experimental study. The lesions observed histologically in various organs of sacrificed rats were as follows.

4.7.2.1 Thyroid Gland

4.7.2.1.1 Group I (Fipronil alone)

Significant histopathological changes could be observed in all the animals examined. The lesions varied from focal degeneration to atrophy and hyper plastic changes. The acini in most cases appeared smaller than normal with reduced amount of colloid. In some the colloid present showed peripheral scalloping (Fig 18). In some other areas hyper plastic acinar cells with tall large nuclei were seen amidst small acini (Fig 19). In some the acinar cells appeared crowded on one side or found filling the entire lumen. There was cystic dilatation of some of the acini (Fig 20). Shrinkage, fibrosis and collapse of some of the acini with a sparse amount of colloid appeared in one or two cases. Follicular cell hypertrophy appeared in one or two cases. Desquamations of lining epithelial cells into lumen forming clumps were seen in a few acini.

4.7.2.1.2 Group *II (Fipronil and curcumin group)*

Follicles with acini of uniform size filled with colloid could be observed (Fig 21). Some of the acini appeared devoid of colloid and scalloping of colloid could also be seen. Lining cells appeared cuboidal with large nuclei in most of the acini. In one or two cases, follicular atrophy with follicular fibrosis and acinar collapse were observed (Fig 22).

4.7.2.2 Liver

4.7.2.2.1 *Group I*

In majority of the cases, lesions varying from mild degenerative changes to diffuse necrosis were observed. In some cases, extensive central venous congestion, sinusoidal congestion, vacuolation of hepatocytes and necrosis with characteristic cytoplasmic and nuclear changes were observed (Fig 23). Hypertrophy and individualization of hepatocytes were also observed.

4.7.2.2.2 *Group II*

The parenchyma of the liver appeared normal and the cells were seen well distributed in the normal architectural pattern around the central vein in most of the cases. Some of the hepatocytes appeared bi-nucleated. There was prominent Kupffer cell reaction and basophillia of hepatocytes surrounding the area of damaged hepatocytes (Fig 24). In a few cases central venous congestion (CVC) and sinusoidal congestion could be seen.

4.7.2.3 Kidney

4.7.2.3.1 Group I

Degeneration and necrosis of the tubules and glomeruli were seen in some cases In five cases there was diffuse haemorrhages in the intertubular areas. Glomerular shrinkage, multifocal congestion and disorganized tubular epithelial cells were also seen (Fig 25 and 26).

4.7.2.3.2 Group II

Most of the tubules and glomeruli appeared normal with usual cellularity (Fig 27). Mild degeneration of tubules and medullary haemorrhages were observed in few a cases (Fig 28).

4.7.2.4 Lung

4.7.2.4.1 Group I

Multifocal to diffuse congestion, alveolar septal thickening, necrosis of alveolar cells and focal infiltration of cells were seen in five cases (Fig 29). Peribronchial venous stasis, focal atelectsis, hemorrhage and oedema were also observed in the two rats. One rat showed lesions such as peribronchial lymphoid cell hyperplasia with germinal centre formation (Fig30), brochostenosis with cell debris and peribronchiloar mononuclear accumulation.

4.7.2.4.2 Group II

Two cases showed generalized congestion and focal aggregation of mononuclear cells (Fig 31). Lungs of seven rats were microscopically normal in appearance.

4.7.2.5 Heart

4.7.2.5.1 Group I

One case showed diffuse vacuolation of myocardial fibers and mild haemorrhage. Focal myocytolysis and hyalinization of muscle fibers were seen in three cases (Fig 32) Extensive congestion was evident in all the animals.

4.7.2.5.2 Group II

Congestion and mild focal hemorrhage were noticed in one case.

4.7.2.6 Brain

4.7.2.6.1 Group I

There was moderate meningeal congestion. Neurons and glial cells appeared intact in the cerebral cortical areas and the glial cell population was normal (Fig 33).

4.7.2.6.2 Group II

There was definitely an increase in the glial cell response (astrocytosis) in the cerebral cortical areas of all the animals (Fig 34). The neurons appeared intact and the white matter appeared without changes.

4.7.2.7 Spleen

4.7.2.7.1 Group I

Congestion and haemosiderosis could be seen in almost all the animals. Few animals revealed predominance of red pulp and vacuolation of splenocytes (Fig 35).

4.7.2.7.2 Group II

Congestion and predominance of white pulp were the major histopathological changes observed (Fig 36).

4.7.2.8 Intestine

4.7.2.8.1 Group I

Goblet cell hyperplasia, desquamation and fusion of villi were seen in most of the cases (Fig 37).

4.7.2.8.2 Group II

Glandular epithelial hyperplasia along with goblet cell predominance was observed in one or two case (Fig 38).

4.7.2.9 Stomach

4.7.2.9.1 Group I

Hyperkeratosis of the nonglandular stomach along with dilatation of gland could be seen in a few animals.

4.7.2.9.2 Group II

A mild glandular hyperplasia could be seen in one or two cases.

4.7.2.2 Group III (control)

Thyroid (Fig.17), liver, kidney, lung, stomach, intestine, spleen and brain on microscopical examination did not reveal any changes.

Table 1: Mean body weight (g) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	BODY WEIGHT (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$186.30^{a} \pm 0.59$	$193.36^{a} \pm 1.18$	$201.11^{a} \pm 1.67$	$199.60^{a} \pm 0.72$	$211.26^{a} \pm 2.18$
II	$187.18^{a} \pm 1.18$	$198.81^{b} \pm 1.81$	$207.42^{b} \pm 1.17$	$214.24^{b} \pm 1.03$	$220.13^{b} \pm 1.73$
III	$185.83^{a} \pm 1.04$	$205.04^{c} \pm 1.17$	$213.64^{c} \pm 0.95$	$221.23^{c} \pm 1.38$	$232.31^{c} \pm 1.88$

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

(a, b, c - Shows significance at five per cent level)

Table 2: Mean levels of total protein (g/dl) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	TOTAL PROTEIN (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$6.51^{a} \pm 0.22$	$6.01^{a} \pm 0.10$	$5.61^{a} \pm 0.06$	$5.18^{a} \pm 0.08$	$4.91^{a} \pm 0.08$
II	$6.46^{a} \pm 0.13$	$6.36^{b} \pm 0.10$	$6.24^{b} \pm 0.14$	$6.17^{b} \pm 0.10$	$6.04^{b} \pm 0.12$
III	$6.42^{a} \pm 0.10$	$6.37^{b} \pm 0.05$	$6.36^{b} \pm 0.12$	$6.41^{b} \pm 0.11$	$6.47^{c} \pm 0.11$

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

(a, b, c - Shows significance at five per cent level)

Table 3: Mean levels of albumin (g/dl) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	ALBUMIN (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$3.61^{a} \pm 0.15$	$3.55^{a} \pm 0.08$	$3.00^{a} \pm 0.12$	$2.86^{a} \pm 0.08$	$2.74^{a} \pm 0.10$
II	$3.67^{a} \pm 0.07$	$3.58^{a} \pm 0.12$	$3.46^{b} \pm 0.07$	$3.42^{b} \pm 0.13$	$3.25^{b} \pm 0.11$
III	$3.59^{a} \pm 0.08$	$3.62^{a} \pm 0.13$	$3.58^{b} \pm 0.10$	$3.62^{b} \pm 0.04$	$3.64^{c} \pm 0.08$

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

(a, b, c - Shows significance at five per cent level)

Table 4: Mean levels of globulin (g/dl) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	Globulin(g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$2.90^{a} \pm 0.27$	$2.46^{a} \pm 0.10$	$2.61^{a} \pm 0.11$	$2.32^{a} \pm 0.09$	$2.17^{a} \pm 0.12$
II	$2.79^{a} \pm 0.16$	$2.78^{a} \pm 0.12$	$2.78^{a} \pm 0.15$	$2.75^{b} \pm 0.10$	$2.79^{b} \pm 0.15$
III	$2.83^{a} \pm 0.13$	$1.75^{a} \pm 0.14$	$2.78^{a} \pm 0.14$	$2.79^{b} \pm 0.08$	$2.83^{b} \pm 0.14$

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

(a, b - Shows significance at five per cent level)

Table 5: Mean levels of aspartate aminotransferase (U/L) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	ASPARTATE AMINOTRANSFERASE(U/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$181.18^{a} \pm 6.26$	$201.68^{a} \pm 3.46$	$226.26^{a} \pm 2.5$	$229.88^{a} \pm 1.63$	$258.24^{a} \pm 1.32$
II	$180.58^{a} \pm 4.36$	$192.96^{b} \pm 3.65$	$189.3^{a} \pm 2.86$	$193.28^{b} \pm 1.49$	$199.82^{b} \pm 1.23$
III	$182.24^{a} \pm 1.46$	$181.26^{b} \pm 1.44$	$183.16^{b} \pm 2.34$	$182.68^{c} \pm 1.34$	$181.56^{c} \pm 1.09$

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

(a, b, c - Shows significance at five per cent level)

Table 6: Mean levels of alanine aminotransferase (U/L) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	ALANINE AMINOTRANSFERASE(U/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$62.14^{a} \pm 1.07$	$86.78^{a} \pm 0.65$	$93.28^{a} \pm 0.76$	$98.6^{a} \pm 2.41$	$102.26^{a} \pm 3.4$
II	$60.88^{a} \pm 1.02$	$69.16^{b} \pm 0.94$	$72.48^{b} \pm 0.90$	$75.74^{b} \pm 2.43$	$78.26^{b} \pm 0.97$
III	$62.62^{a} \pm 0.77$	$63.72^{c} \pm 0.58$	$62.86^{c} \pm 0.68$	$65.16^{c} \pm 1.18$	$63.16^{c} \pm 0.83$

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

(a, b, c - Shows significance at five per cent level)

Table 7: Mean levels of cholesterol (mg/dl) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	CHOLESTEROL(mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$49.18^{a} \pm 1.11$	$49.49^{a} \pm 1.20$	$50.23^{a} \pm 1.07$	$51.18^{a} \pm 0.57$	$52.34^{a} \pm 0.54$
II	$49.75^{a} \pm 0.51$	$48.41^{a} \pm 0.50$	$48.10^{a} \pm 0.99$	$47.68^{b} \pm 0.58$	$47.50^{b} \pm 0.79$
III	$50.50^{a} \pm 0.67$	$51.25^{a} \pm 0.60$	$49.82^{a} \pm 0.84$	$49.75^{b} \pm 0.47$	$50.25^{b} \pm 0.76$

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

(a, b - Shows significance at five per cent level)

Table 8: Mean levels of creatinine (mg/dl) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	CREATININE(mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$0.62^{a} \pm 0.05$	$0.64^{a} \pm 0.06$	$0.65^{a} \pm 0.05$	$0.72^{a} \pm 0.04$	$0.85^{a} \pm 0.04$
II	$0.61^{a} \pm 0.07$	$0.62^{a} \pm 0.05$	$0.59^{a} \pm 0.03$	$0.65^{a} \pm 0.02$	$0.64^{b} \pm 0.05$
III	$0.63^{a} \pm 0.04$	$0.61^{a} \pm 0.04$	$0.61^{a} \pm 0.04$	$0.62^{a} \pm 0.03$	$0.63^{b} \pm 0.02$

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

(a, b - Shows significance at five per cent level)

Table 9 : Mean packed cell volume (%) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	PACKED CELL VOLUME (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$45.64^{a} \pm 0.53$	$45.09^{a} \pm 0.49$	$43.30^{a} \pm 0.68$	$42.97^{a} \pm 0.58$	$42.11^{a} \pm 0.53$
II	$45.34^{a} \pm 0.49$	$44.34^{a} \pm 0.69$	$44.13^{b} \pm 0.39$	$44.19^{b} \pm 0.43$	$43.90^{b} \pm 0.56$
III	$45.01^{a} \pm 0.55$	$44.96^{a} \pm 0.64$	$45.29^{b} \pm 0.47$	$45.15^{b} \pm 0.48$	$45.37^{b} \pm 0.42$

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

(a, b, - Shows significance at five per cent level)

Table 10: Mean haemoglobin concentration (g/dl) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	HAEMOGLOBIN CONCENTRATION (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$15.14^{a} \pm 0.23$	$15.16^{a} \pm 0.25$	$14.90^{a} \pm 0.48$	$14.76^{a} \pm 0.31$	$14.21^{a} \pm 0.35$
II	$15.34^{a} \pm 0.26$	$15.12^{a} \pm 0.33$	$15.02^{a} \pm 0.40$	$15.11^{a} \pm 0.31$	$15.02^{b} \pm 0.39$
III	$15.10^{a} \pm 0.25$	$15.05^{a} \pm 0.45$	$15.46^{a} \pm 0.32$	$15.38^{a} \pm 0.26$	$15.43^{b} \pm 0.36$

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

(a, b - Shows significance at five per cent level)

Table 11: Mean erythrocyte sedimentation rate (mm per one hour) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	ERYTHROCYTE SEDIMENTATION RATE(Millimeter per one hour)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$0.86^{a} \pm 0.10$	$0.98^{a} \pm 0.06$	$0.92^{a} \pm 0.04$	$0.94^{a} \pm 0.05$	$0.90^{a} \pm 0.05$
II	$0.90^{a} \pm 0.11$	$0.88^{a} \pm 0.07$	$0.86^{a} \pm 0.02$	$0.92^{a} \pm 0.03$	$0.88^{a} \pm 0.07$
III	$0.88^{a} \pm 0.07$	$0.92^{a} \pm 0.05$	$0.90^{a} \pm 0.10$	$0.90^{a} \pm 0.01$	$0.92^{a} \pm 0.04$

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

Table 12: Mean total leukocyte count (thousands per mm^3) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	TOTAL LEUKOCYTE COUNT(Thousands per mm^3)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$8.69^{a} \pm 0.06$	$8.68^{a} \pm 0.12$	$8.79^{a} \pm 0.09$	$8.76^{a} \pm 0.11$	$8.77^{a} \pm 0.08$
II	$8.71^{a} \pm 0.09$	$8.62^{a} \pm 0.12$	$8.71^{a} \pm 0.08$	$8.71^{a} \pm 0.08$	$8.74^{a} \pm 0.09$
III	$8.68^{a} \pm 0.06$	$8.64^{a} \pm 0.11$	$8.70^{a} \pm 0.11$	$8.73^{a} \pm 0.11$	$8.72^{a} \pm 0.09$

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

Table 13: Mean Differential Leukocyte Count-lymphocytes (%) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	DIFFERENTIAL LEUKOCYTE COUNT-LYMPHOCYTES (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$80.75^{a} \pm 1.12$	$81.90^{a} \pm 1.20$	$81.94^{a} \pm 1.04$	$81.93^{a} \pm 0.92$	$82.11^{a} \pm 1.00$
II	$80.60^{a} \pm 0.87$	$80.80^{a} \pm 0.90$	$80.86^{a} \pm 0.94$	$80.97^{a} \pm 0.96$	$80.28^{a} \pm 0.92$
III	$80.20^{a} \pm 0.92$	$80.61^{a} \pm 0.80$	$80.35^{a} \pm 1.11$	$80.36^{a} \pm 0.97$	$80.13^{a} \pm 0.58$

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

Table 14: Mean Differential Leukocyte Count- Neutrophils (%) of rats [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

Groups	DIFFERENTIAL LEUKOCYTE COUNT-NEUTROPHILS (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$19.25^{a} \pm 1.12$	$18.10^{a} \pm 1.20$	$18.05^{a} \pm 1.04$	$18.07^{a} \pm 0.92$	$17.89^{a} \pm 1.0$
II	$19.40^{a} \pm 0.87$	$19.20^{a} \pm 0.90$	$19.14^{a} \pm 0.94$	$19.03^{a} \pm 0.96$	$19.72^{a} \pm 0.92$
III	$19.80^{a} \pm 0.92$	$19.38^{a} \pm 0.80$	$19.64^{a} \pm 1.11$	$19.64^{a} \pm 0.97$	$19.88^{a} \pm 0.58$

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

Table 15: Mean levels of lipid peroxides (nMol/g), Reduced Glutathione (µg/g) and Superoxide Dismutase (Units /mg of protein) of rat liver [Group I (Fipronil alone), Group II (Fipronil and curcumin) and Group III (Control)]

GROUPS	LIPID PEROXIDES (nMol/g)	REDUCED GLUTATHIONE (µg/g)	SUPEROXIDE DISMUTASE(Units /mg of protein)
I	$452.44^{a} \pm 15.58$	$435.35^{a} \pm 6.34$	$42.36^{a} \pm 0.85$
II	$336.27^{b} \pm 8.37$	$513.85^{b} \pm 4.49$	$48.82^{b} \pm 0.58$
III	$325.06^{b} \pm 6.26$	$526.39^{b} \pm 5.5$	$50.24^{b} \pm 0.90$

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

(a, b - Shows significance at five per cent level)

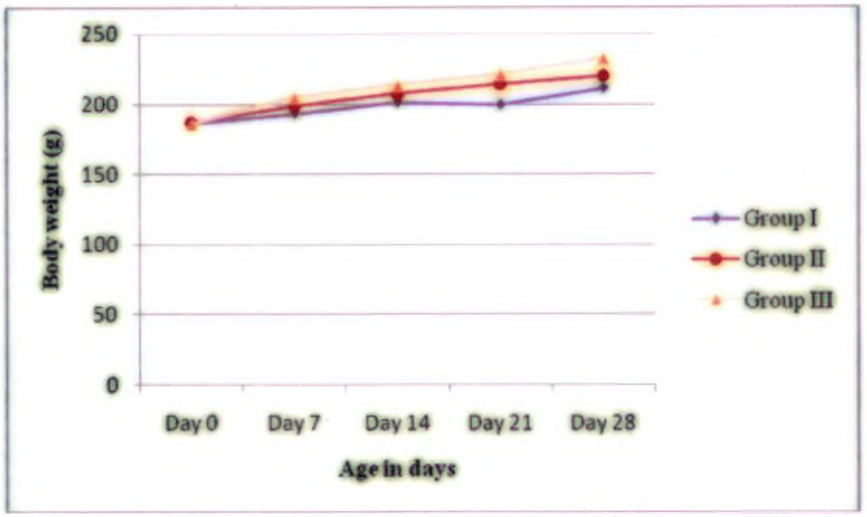

Fig.1 Mean body weights (g) of animals

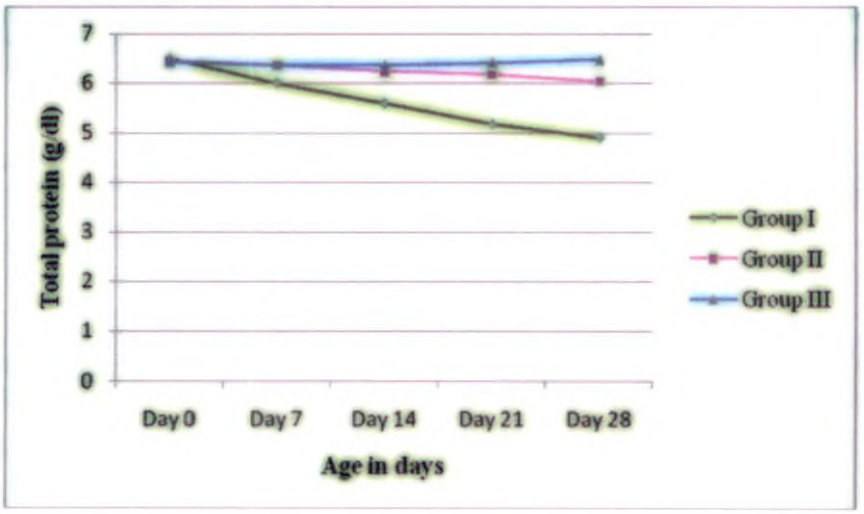

Fig.2 Mean total protein levels (g/dl)

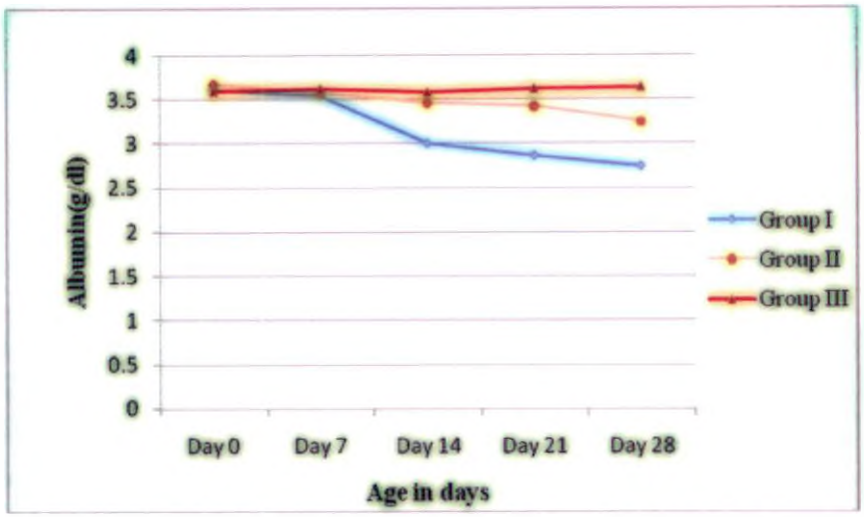

Fig.3 Mean albumin levels (g/dl)

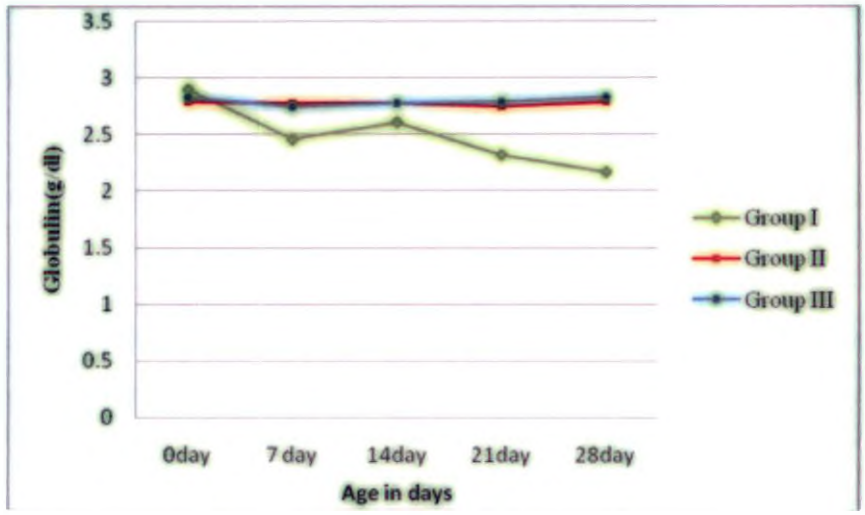

Fig. 4 Mean globulin levels (g/dl)

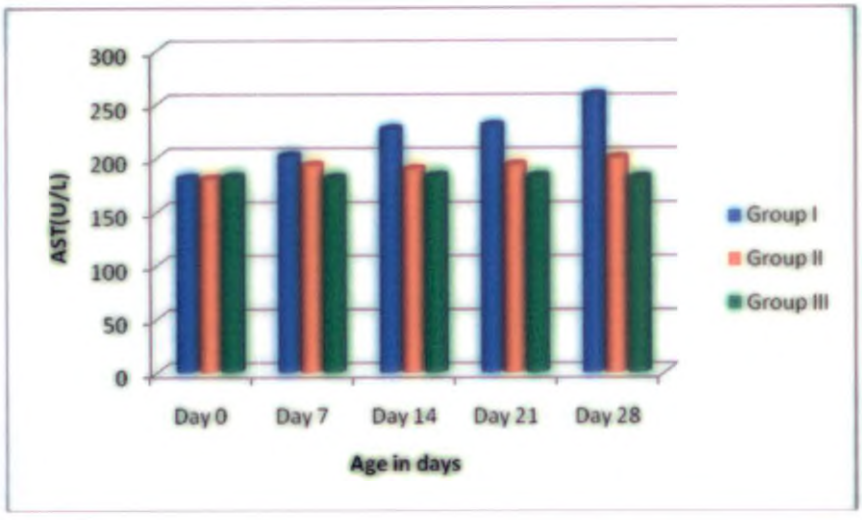

Fig. 5 Mean Aspartate aminotransferase levels (U/L)

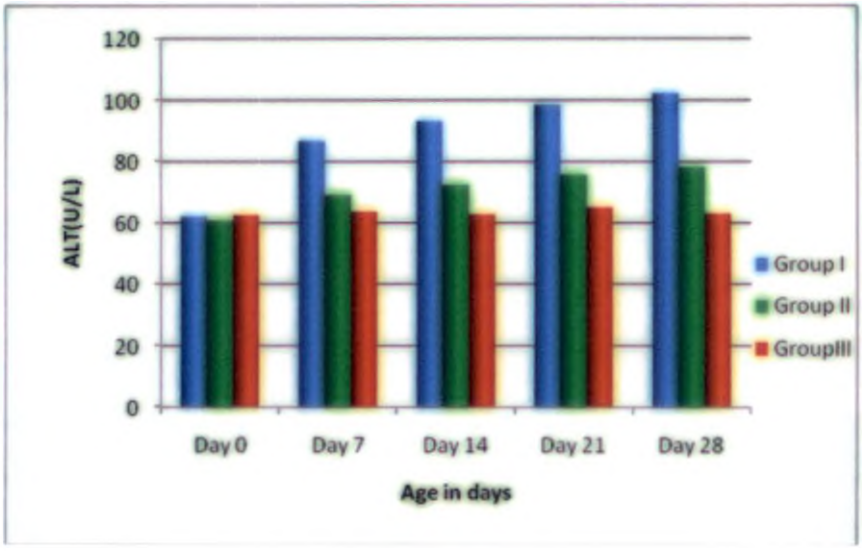

Fig. 6 Mean Alanine aminotransferase levels (U/L)

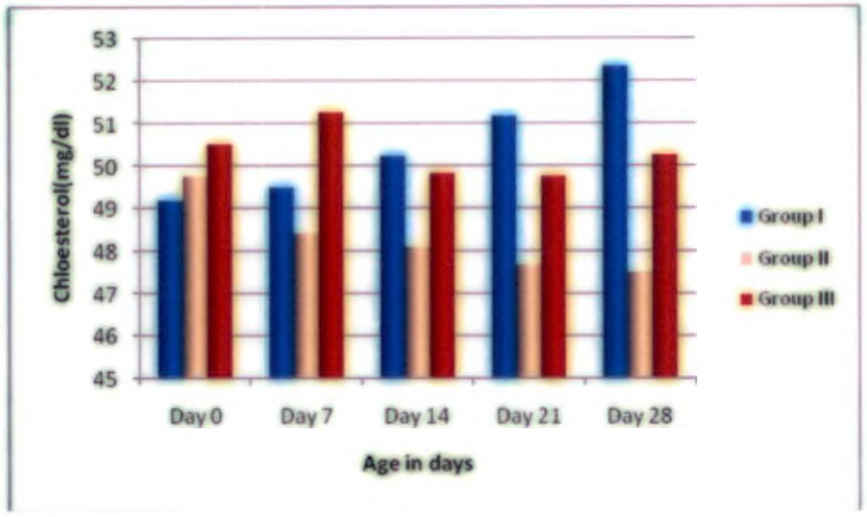

Fig.7 Mean Cholesterol levels (mg/dl)

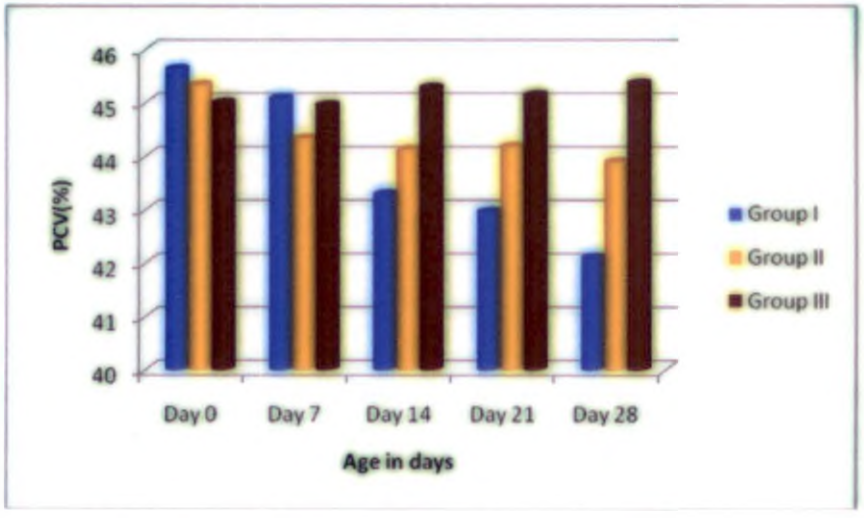

Fig. 8 Mean Packed cell volume (percentage)

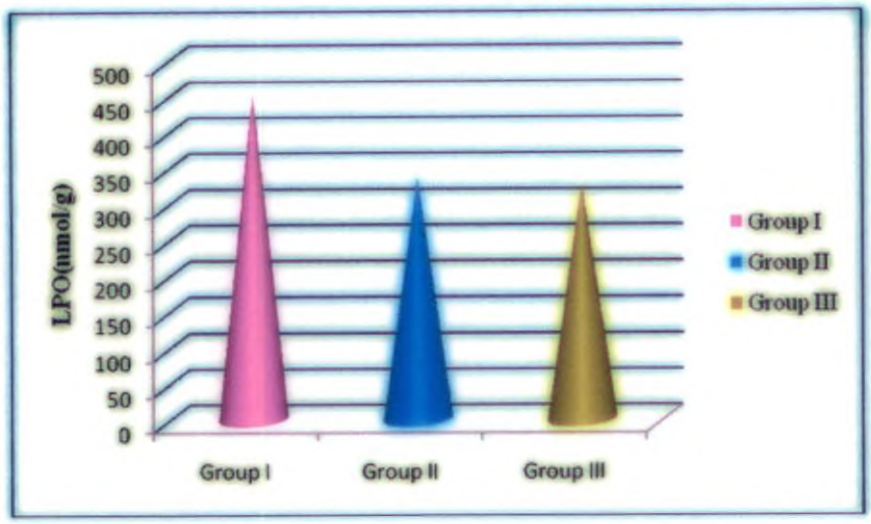

Fig. 9 Mean levels of lipid peroxides in liver (nmol/g)

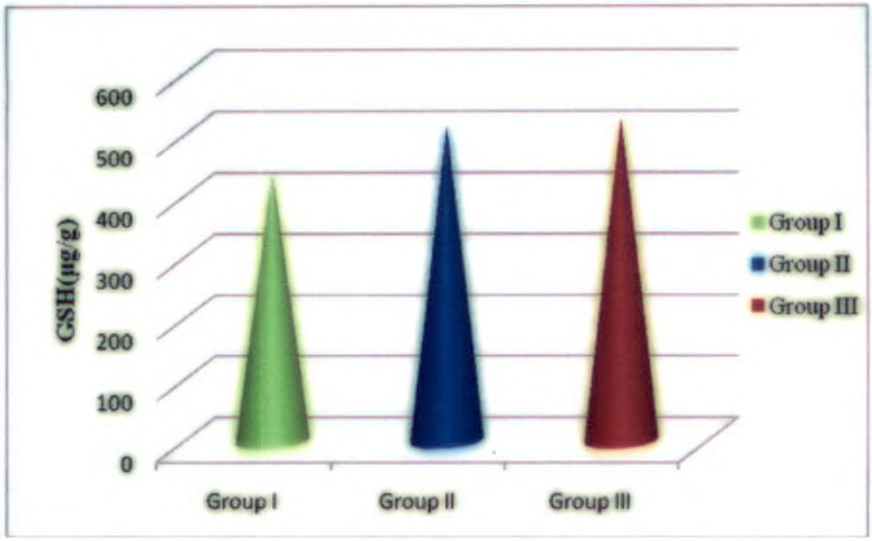

Fig. 10 Mean levels of reduced glutathione in liver (µg/g)

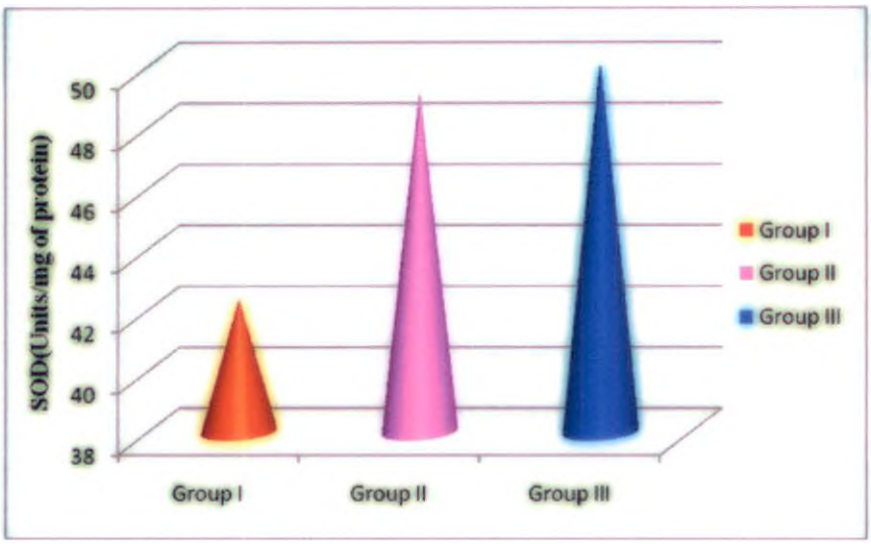

Fig. 11 Mean level of superoxide dismutase in liver (Units/mg of protein)

Fig. 12 Group I (fipronil alone)-Dullness and depression

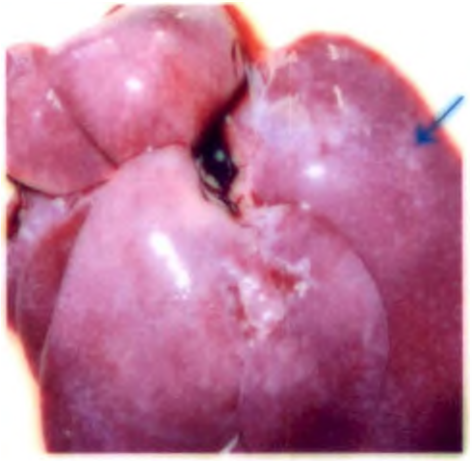

Fig.13

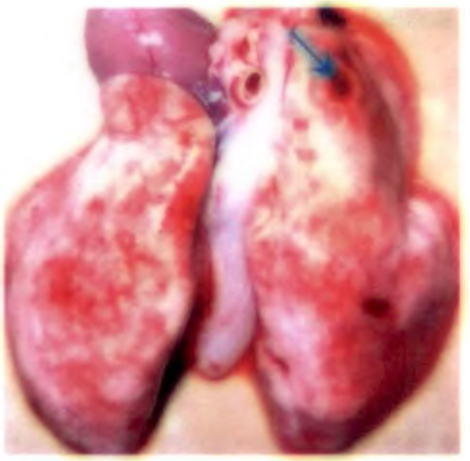

Fig.14

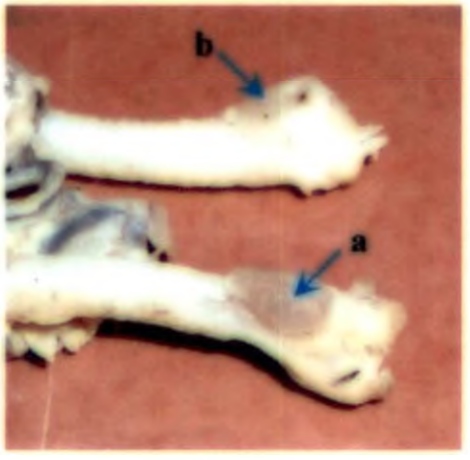

Fig.15

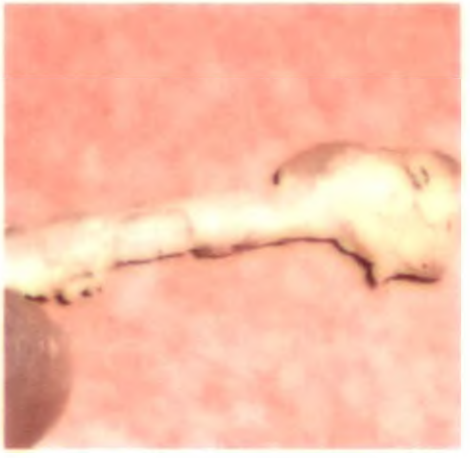

Fig.16

Fig. 13 Group I (fipronil alone) Liver- hepatomegaly and greyish white spots of necrosis

Fig. 14 Group I - Lung – Congestion and haemorrhage

Fig. 15 Group I – Enlarged thyroid (a) group III (control)-normal thyroid (b)

Fig. 16 Group II (fipronil and curcumin)-Thyroid-moderately enlarged

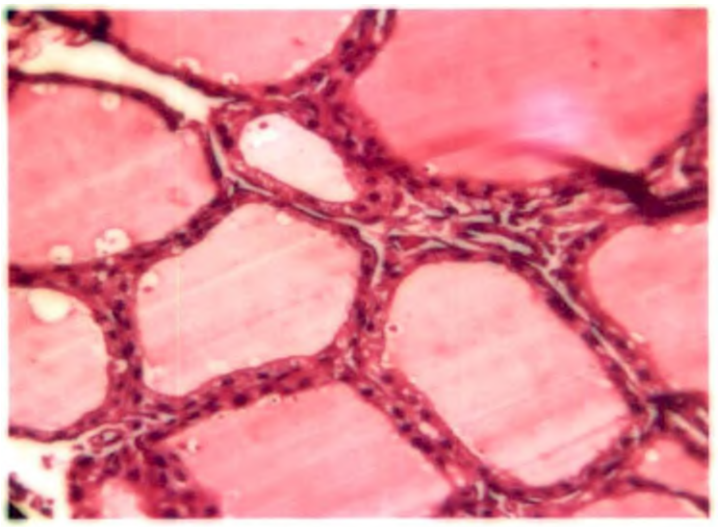

Fig .17 Group III (control)-Thyroid –Normal H&E × 400

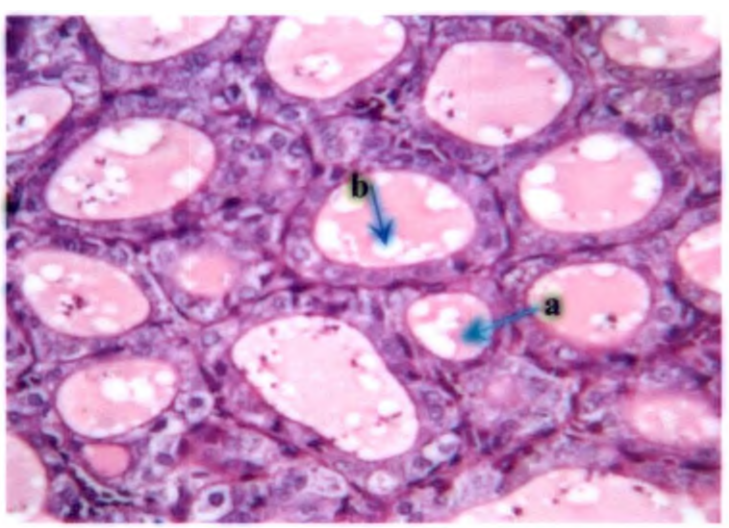

Fig.18 Group I -Thyroid- – Smaller acini (a) and Peripheral scalloping of colloid (b) H&E × 400

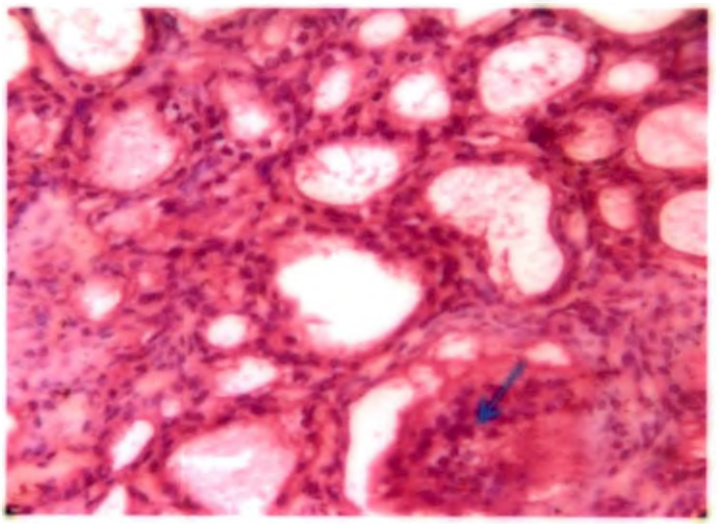

Fig.19 Group I -Thyroid hyperplasia, H&E × 400

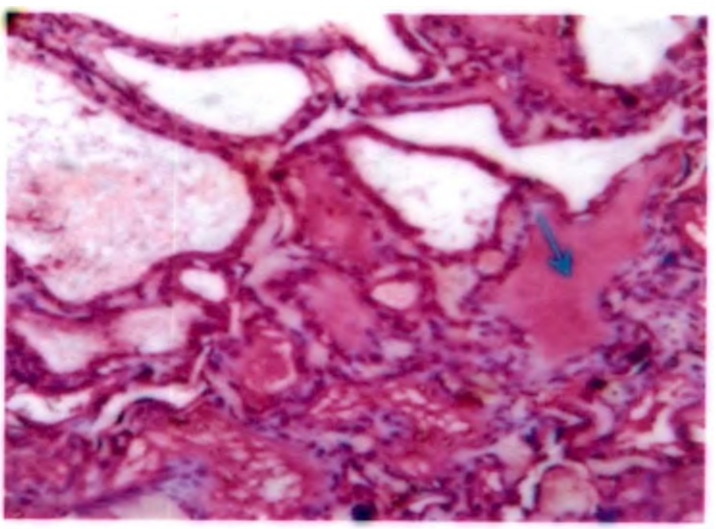

Fig.20 Group I- Thyroid- hyperplasia and cystic dilatation of some acini, H&E × 400

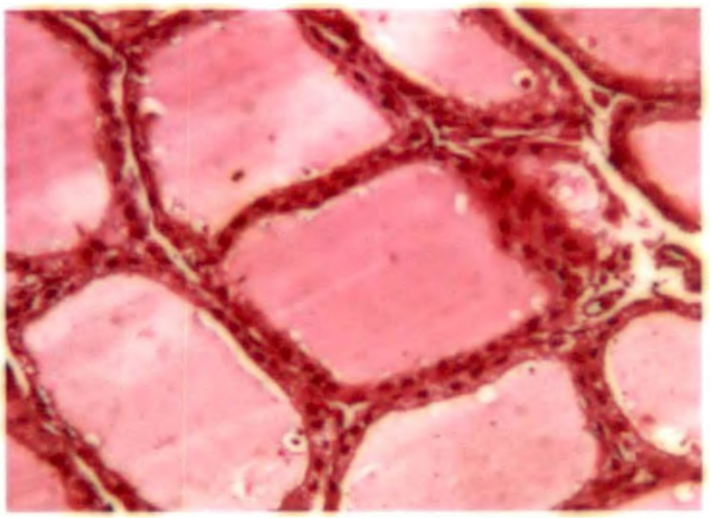

Fig. 21 Group II (Fipronil and curcumin)- Thyroid- Uniform sized follicular acini, H&E × 400

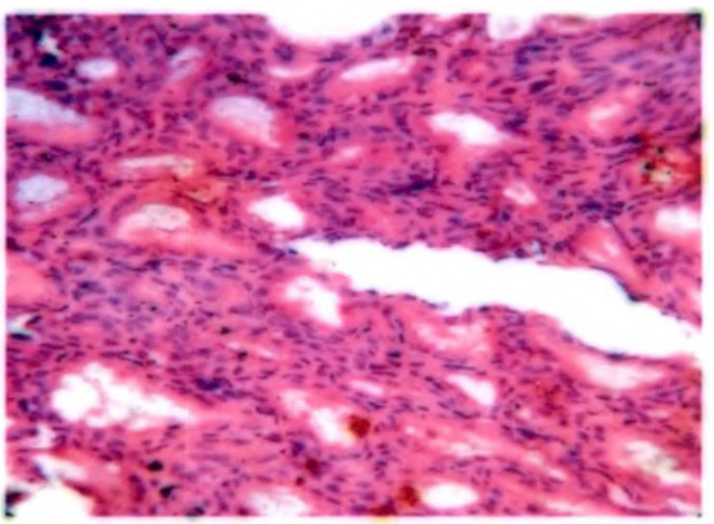

Fig. 22 Group II- Thyroid – follicular atrophy and fibrosis, H&E × 400

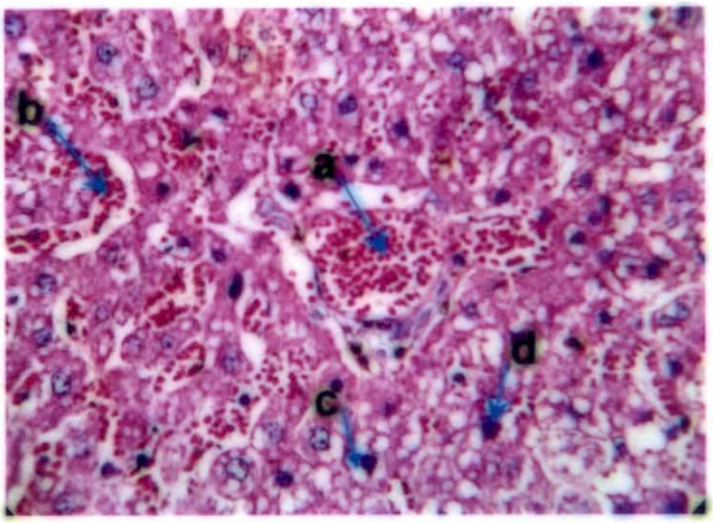

Fig. 23 Group I -Liver- Extensive central venous congestion(a), sinusoidal congestion (b), vacuolation (c) and necrosis of hepatocytes (d), H&E × 400

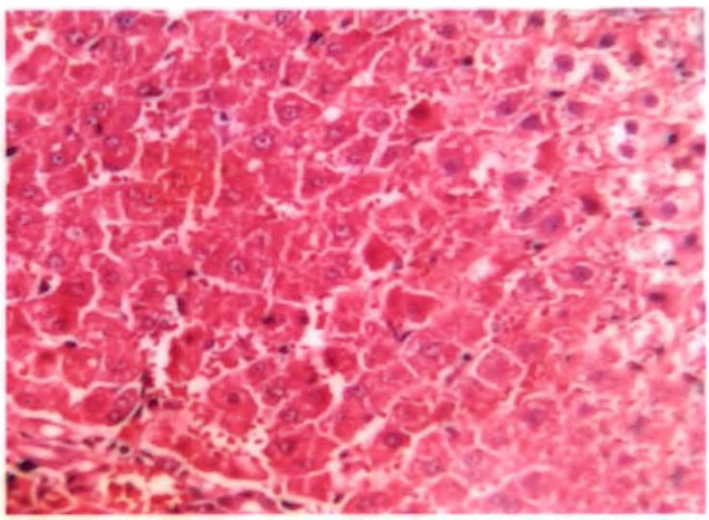

Fig 24 Group II -Liver- kupffer cell reaction and basophilic hepatocytes around the area of damage, H&E × 400

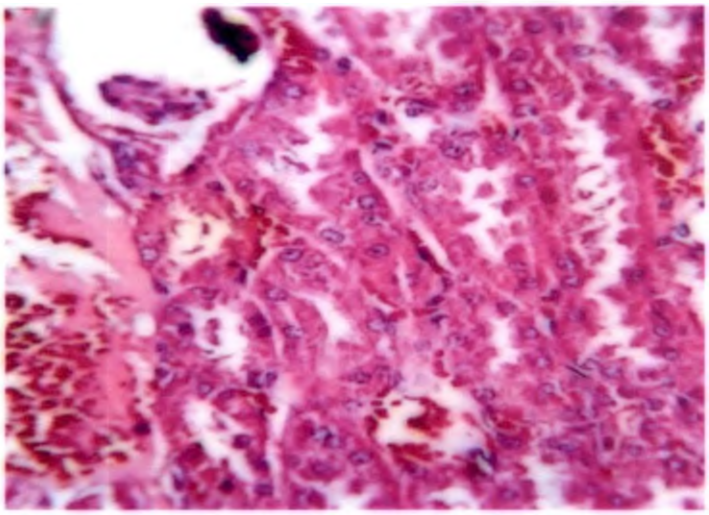

Fig. 25 Group l- Kidney-degeneration and necrosis of tubules and glomeruli, H&E × 400

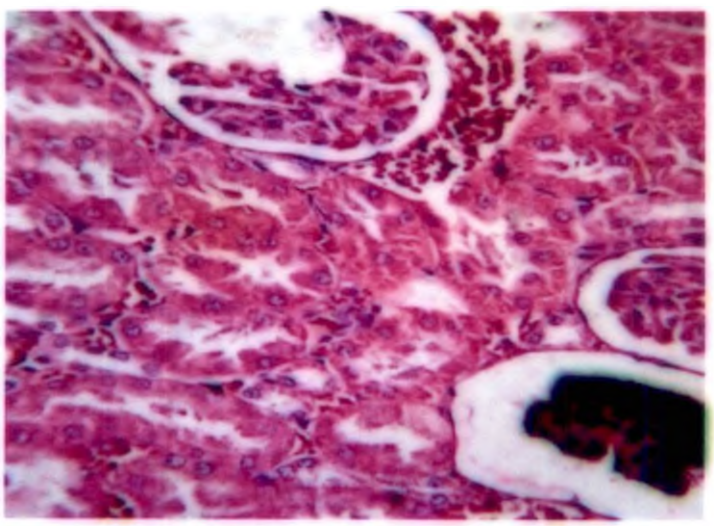

Fig. 26 Group l -Kidney- shrinkage and necrosis of glomeruli, H&E × 400

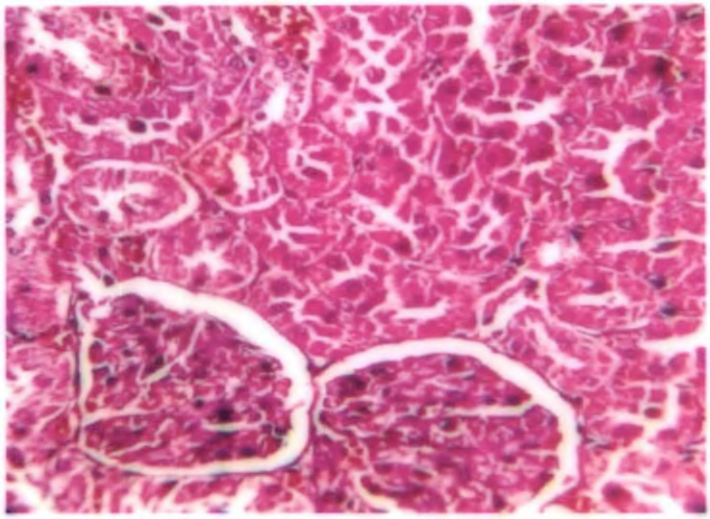

Fig. 27 Group II -Kidney – Normal tubules and glomeruli H&E × 400

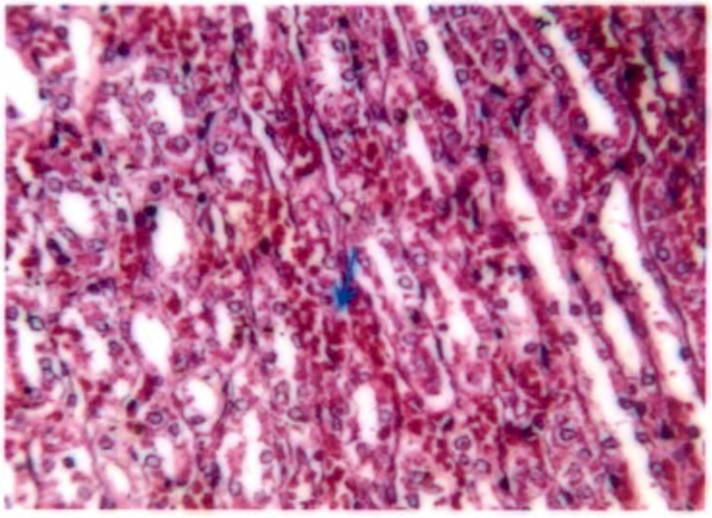

Fig. 28 Group II- Kidney – Mild tubular degeneration and medullary haemorrhage, H&E × 400

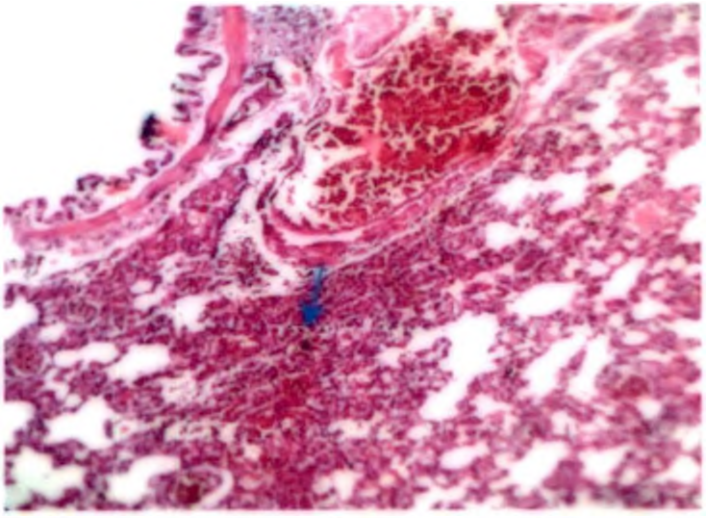

Fig.29 Group I- Lung –Diffuse congestion, alveolar septal thickening and necrosis of alveolar cells, H&E × 100

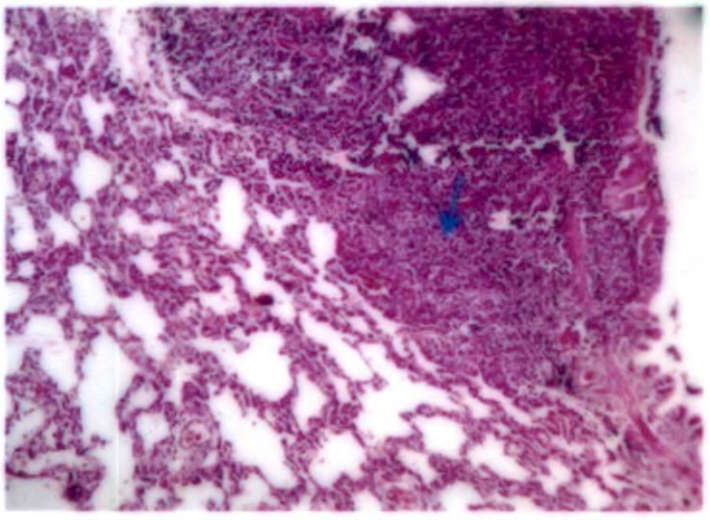

Fig. 30 Group I- Lung-Peribrochial lymphoid cell hyperplasia H&E × 100

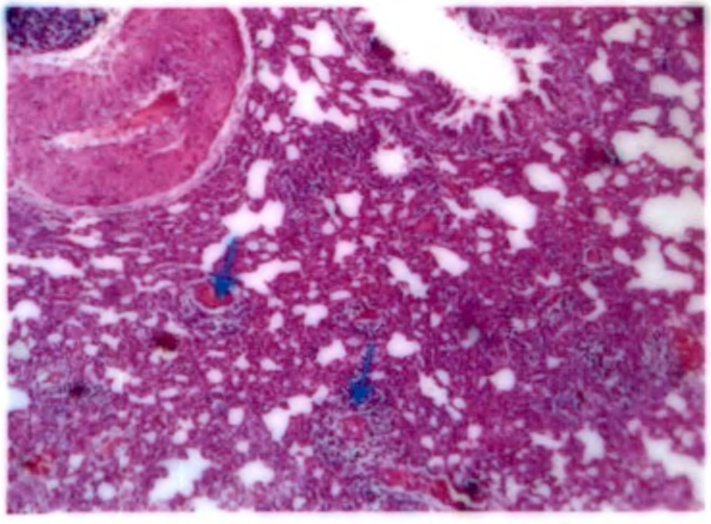

Fig. 31 Group II- Lung- Congestion and focal mono nuclear cell aggregation, H&E × 100

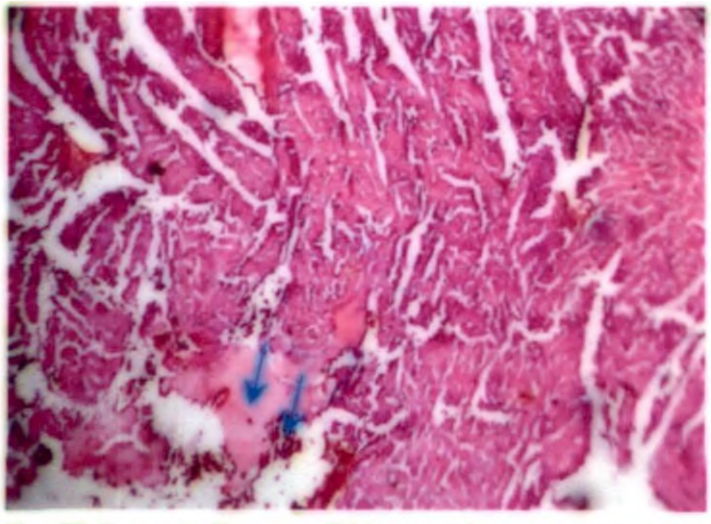

Fig. 32 Group I- Heart - mild haemorrhage, myocytolysis and hyalinization, H&E × 100

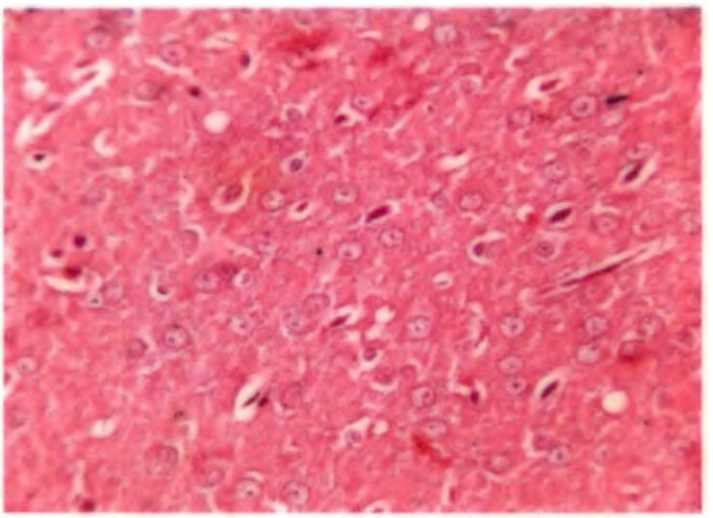

Fig. 33 Group I - Brain - normal glial cell population, H&E × 400

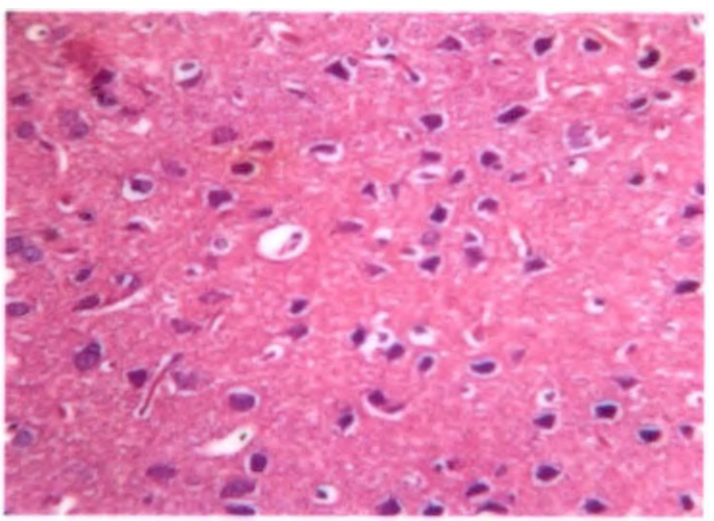

Fig. 34 Group II -Brain- glial cell response, H&E × 400

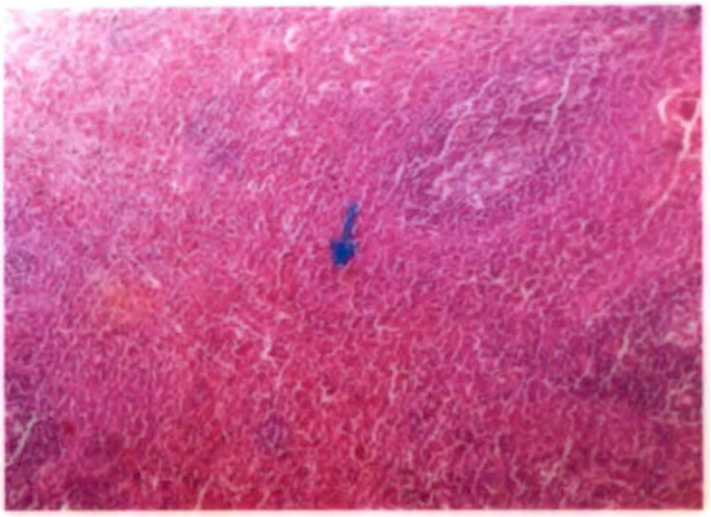

Fig. 35 Group I -Spleen- Red pulp predominance, H&E × 100

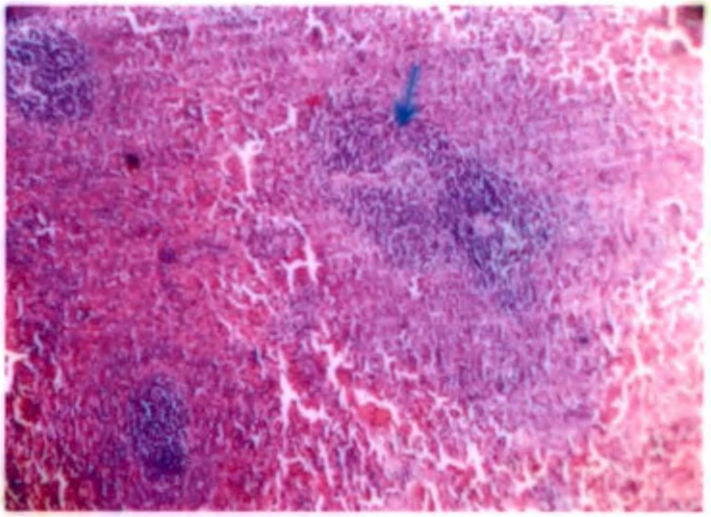

Fig. 36 Group II -Spleen-White pulp predominance, H&E × 100

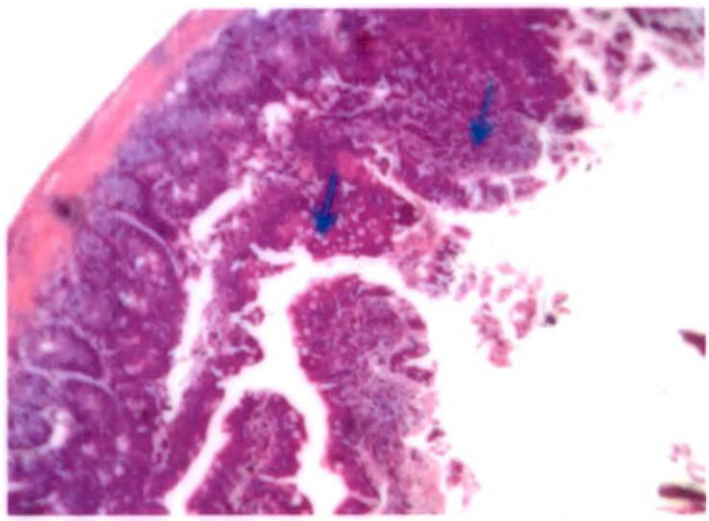

Fig. 37- Group I Intestine-Goblet cell hyperplasia, desquamation and fusion of villi, H&E × 100

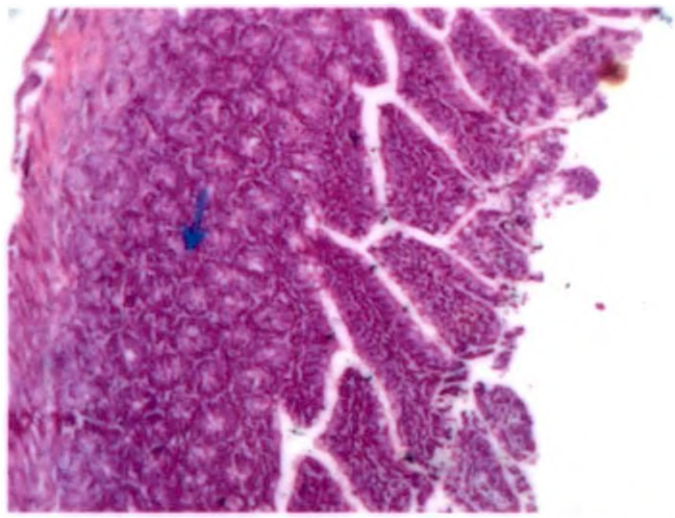

Fig. 38- Group II Intestine - glandular and goblet cell hyperplasia, H&E × 100

Discussion

5. DISCUSSION

Fipronil a phenylpyrazole derivative of new generation insecticide has been introduced into market since 1993. It is an insect growth inhibitor which has found place as an ectoparasiticide in animals too. Perusal of literature revealed only limited information on this insecticide and most of the studies were confined to pharmacokinetics, metabolism and certain biochemical effects. Systematic investigations on the general toxicological effects have not been assessed. It is observed that indiscriminate and unscientific application of this resulted in severe ecological problems and health hazards to humans and animals. Hence this study has been formulated to evaluate the toxicopathological, hematobiochemical and membrane damaging effects of fipronil in rats using almost $1/3^{rd}$ of LD_{50} dose and the protective effect of curcumin against the damage if any produced at this dose rate.

5.1 PHYSIOLOGICAL PARAMETERS

5.1.1 Body Weight

Significantly ($p<0.05$) lower mean body weight was observed in both group I and group II compared to group III. The rats in treatment groups didn't gain bodyweight as much as control group as there was inappetence and reduction in feed intake. Fluoride action network (2004) and Department for environment food and rural affairs (2004) observed significant reduction in body weight gain in rats which were fed with technical grade of fipronil. The higher body weight gain observed in group II could be attributed to the protection carried by curcumin against the toxic effect of fipronil. There was not much lesions produced in any of the organs and whatever produced has been corrected in the presence of curcumin. Al-Sultan (2003) observed the higher body weight gain in curcumin fed broilers by its antioxidant properties.

5.2 BIOCHEMICAL PARAMETERS

5.2.1 Total protein, Albumin and Globulin

On day 28th, group I showed significantly lower mean serum total protein and albumin levels compared to group II and group III. Group II showed significantly lower levels compared to group III .On day 14th and 21st, group I showed significantly lower mean serum total protein and albumin levels compared to group II and group III where as group II showed significantly higher total protein and albumin levels compared with group I but lower total protein and albumin levels compared to group III. There was no significant difference between group II and group III. On 21st and 28th days, group I showed significantly lower globulin level compared to group II and group III. The hypoproteinemia and hypoalbuminemia observed in the present study may be attributed to the liver damage caused by the toxic agent. Histologically there was moderate hepatocyte damage. Liver is the only site for albumin synthesis where hypoalbuminemia is an important feature of liver toxicity. Joint meeting of FHO/WHO on pesticidal residues (1997) and Hovda and Hooser (2002) observed significantly lower total serum protein, albumin and globulin levels in rats which were administered with fipronil. Group II showed significantly higher total protein and albumin levels compared to group I which may be attributed to the protective effect of curcumin. Curcumin also might have reduced hepatocytes destruction by its antioxidant properties. Deshpande *et al.* (1998) and Gordillo *et al.* (2007) observed significant increase in total protein and albumin level in rats when they administered with curcumin and CCl4.

5.2.2 Serum Enzymes

The AST level was found increased gradually from day seventh but statistically significant increase was observed in group I on day 7th, 21st and 28th. A gradual

significant increase in the ALT Level was observed in group I on day from day seventh onwards compared to group II and group III. The elevated Enzyme levels may be attributed to pathological changes produced by fipronil on the hepatobiliary sysem which resulted in increased cellular permeability and release of these enzymes into the serum. Fluoride action network (2004) and Wang *et al.* (2008) observed significant increase AST and ALT levels in the serum of rats when they fed with fipronil. Balasubramaniam *et al.* (1998) observed that administration of lindane to rats resulted in significant increase of AST and ALT levels in the serum. The serum level of these enzymes was significantly lower in fipronil and curcumin group for the entire experimental period compared to fipronil group alone. The decreasing trend of AST and ALT levels in group II can be attributed to the hepatoprotective and antioxidant properties of curcumin against the pathological changes induced by fipronil. Deshpande *et al.* (1998), Park *et al.* (2000) and Gordillo *et al.* (2007) observed significant reduction in AST and ALT levels when rats were treated with curcumin and CCl_4.

5.2.3 Cholestrol

On 21st and 28th days, a significant increase in the cholesterol level was observed in the group I compared to group II and group III. The hypercholestremia could be attributed to both liver damage and hypothyroidism as there was damage to both these organs. Significant depletion of colloid in the thyroid follicles and hyperplasia of the lining epithelium giving rise to a parenchymatous type of goiter was observed. Joint Meeting of FAO/WHO Pesticidal Residue (1997) and Department of environment food and rural affairs (2004) reported significant increase in the cholesterol level of rats which were fed to different doses of fipronil and its metabolite. Omurtag *et al.* (2008) observed significant increase in cholesterol level in rats when administered with endosulfan. Anon (2004) observed decreased cholesterol level in dogs when they were fed with technical grade of fipronil. The cholesterol

level was found decreasing gradually from seventh day in group II but statistically significant decrease in cholesterol level was observed on days 21st and 28th compared to group I. This could be attributed to curcumin treatment which has hypocholestremic effect. Deshpande *et al.* (1998) observed decrease in serum cholesterol level when rats were administered with CCl_4 and curcumin.

5.2.4 Creatinine

On day 28th, group I showed significantly higher mean creatinine compared to group II and group III. Group II showed significantly lower creatinine level compared to group I. No significant difference was observed between group II and group III. Increased creatinine levels in the serum indicated kidney damage. Degeneration and necrosis of the tubules and glomeruli could be seen in histopathological examination. Hovda and Hooser (2002) observed significant higher elevation of creatinine in rats which were fed with fipronil at 26mg/kg for 90days. Here the experiment was for a period of 28 days and the dose rate was 30.4mg/kg which was definitely high as compared to the above.

5.3 HAEMATOLOGICAL PARAMETERS

Significant variation was not observed in parameters like TLC, DLC and ESR in group I and group II and the values were comparable to group III. This observation is contrary to the findings of fluoride action network (2004).This organization observed significant increase in neutrophils upto 30 percent and total leukocyte count upto 28 per cent in mice when treated with fipronil for 45days. Anon (2004) observed that dietary administration of fipronil to beagle dogs for 52 weeks resulted in singnificant increase in the PCV, Hb concentration and total erythrocyte counts. The duration of exposure and the dose and the species susceptibility have major influence on the hematology. On days 14th, 21st and 28th, group I showed significant decrease in the mean packed cell volume (PCV) compared to control group. Meanwhile

significant decrease in the hemoglobin (Hb) value was observed in group I on day 28th compared to control group. The decreasing trend of PCV and Hb values can be attributed to decreased production of globin and defective storage of iron due to fipronil induced liver damage. Remya (2008) observed significant reduction in the mean PCV and Hb values when rats were administered with cypermethrin. The group II showed significant increase in the PCV and Hb values in these days which could be attributed to the hepatoprotective property of curcumin. Similar changes in PCV and Hb values were reported by Al-Sultan (2003) and Simi (2007) in turmeric (curcumin) fed broilers.

5.4 CLINICAL SIGNS

5.4.1 Group I

Two animals showed dullness, anorexia, abnormal gait, convulsion and aggressiveness in fipronil intoxicated group before their death. Fipronil might have blocked GABA receptors resulting into stimulatory effect of central nervous system. Similar observations were made by world health organization (2000) and Anon (2004) in fipronil toxicity on rats. United State Environment Protection Agency (1998) and Ikeda *et al.* (2001) observed tonic-clonic convulsions and aggressiveness when rats were administered with fipronil and its metabolite. No signs were observed in the survived rats. Parmer *et al.* (2003) observed hyperexcitability and convulsions on third day when rats were exposed to different doses of lindane through oral administration. Kaul *et al.*(1996) observed dullness, tremors, hyper-excitability and convulsions in male rats when administered with fenvalerate, a synthetic pyretheroid at various dose levels.

5.4.2 Group II

One animal showed in appetence and depression before death and the other animals remained healthy and did not show any symptoms. These groups were administered with curcumin and the protection offered by curcumin could be

attributed to the non production of symptoms. Shukla *et al.* (2003) observed decreased neurological signs such as irritability, aggressiveness and coma in lead intoxicated rats when were treated with curcumin. Yadav *et al.* (2009) observed that oral administration of curcumin reduced arsenic neurotoxic signs such as convulsions and hyperexcitability in rats.

5.5 MORTALITY PATTERN

Mortalities were observed in both group I and group II. Twenty percent mortalities were observed in the group I whereas group II showed 10 percent mortality only. Tingle *et al.* (2003) did not observe any significant mortalities in dogs when administered with heavy dose of fipronil. Paul *et al.* (1995) observed 30 percent mortalities when rats were exposed with endosulfan. However, fluoride Action Network (2004) observed heavy mortalities in rats when they were administered with fipronil. The reduction in mortality percentage of group II definitely points to the ameliorative effect of curcumin on the toxicity. Meanwhile Shukla *et al.* (2003) and Yadav *et al.* (2009) did not observe any mortalities in rats which were treated with heavy metals such as arsenic and lead along with curcumin respectively. Curcumin reduced mortalities in the Eimeria affected chicken where curcumin had protective effect against Eimeria spores and oocysts (Allen *et al.*, 1998). No mortalities were observed in group administerd with vehicle honey (Group III).

5.6 OXIDATIVE EFFECTS

Oxidative effect of the fipronil was determined by measuring the alterations in the levels of lipid peroxides, reduced glutathione and superoxide dismutase in liver homogenates. Lipid peroxidation cause severe damage to cell membrane and increased cell fragility thereby causing leakage of intercellular enzyme, malondialdehyde and other compounds resulting in loss of function and cell death (Barros *et al* ., 1991). In this study, it was observed that group I caused oxidative

damage as there was a statistically significant increase in lipid peroxides and a concurrent decrease in reduced glutathione and superoxide dismutase compared to group II and group III. This indicated that fipronil is able to produce oxidative damage to the liver. Histopathological lesions observed in the present study confirmed liver damage in group I. Videla *et al.* (1988) observed higher amount of lipid peroxides and superoxide free radicals concomitant with decrease in reduced glutathione, superoxide dismutase and catalase levels in the liver of rats when they were administered with lindane. Group II showed significant reduction of lipid peroxides levels and an increase in reduced glutathione and superoxide dismutase in the liver. The lower oxidative damage observed in group II could be attributed to the antioxidant activity elicited by curcumin against oxidative damage of fipronil. Histopathological lesions observed in the present study confirmed lesser liver damage in group II compared with group I. Ammon and wahl (1991) opined that curcumin protected oxidative degradation of lipids and hemoglobin in rats. Gordillo *et al.* (2007) found increased level of reduced glutathione and total liver glutathione in rats when they were acutely intoxicated by carbon tetrachloride. Verma and Mathuria (2008) observed increased levels of reduced glutathione, catalase, superoxide dismutase and glutathione peroxidase in the liver and kidney of mice when they were fed to heavy dose of aflatoxin.

5.7 PATHO ANATOMICAL STUDIES

5.7.1 Gross Pathology

Group I animals showed hepatomegaly, congestion and necrosis in the liver and congestion and hemorrhage in the lung. Thyroids were enlarged and hard in consistency. Congestion and haemorrhage were also observed in the kidney. From this study it is found that the target organs of toxicity may be liver and thyroid in fipronil intoxicated rats. This findings correlates with the observations of Hovda and Hooser (2002). The gross lesions of liver observed in this study were in accordance

with those reported by other research workers (Anon, 2004; Wang *et al.*, 2008). Flouride action network (2004) observed enlarged liver, kidney and thyroid in male rats when they fed with fipronil. Junqueira *et al.* (1997) also observed necrotic foci, congestion and haemorrhage in the liver when rats were exposed to heavy dose of lindane. The intensity of these lesions in group II were much less or almost absent compared with group treated with fipronil alone. This could be attributable to the protective effect elicited by curcumin against the toxic effect of fipronil. Gordillo *et al.* (2007) observed greatly reduced gross lesions in the liver when rats were treated with CCl_4 and curcumin. Group III (control) animals did not reveal any macroscopical changes.

5.7.2 Histopathology

Major changes were observed in the thyroid gland. The lesions were smaller acini with reduced amount of colloid, peripheral scalloping of colloid, cystic dilatation of some of the acini, hyperplasia, follicular cell hypertrophy, fibrosis and desquamation of cells in Group I rats which were treated with fipronil alone. An increase in the serum cholesterol level in this group reflected severe damage of thyroid gland. Fluoride action Network (2004) observed thyroid follicular hyperplasia, hypertrophy and congestion in rats when rats were treated with 300 ppm of fipronil. Uniform sized follicular acinus filled with colloid was observed in most of the group II animals. In this group the serum cholesterol level was within normal range or decreased to certain extend compared to control. These rats were protected with curcumin. These observation points to the need for further study on the thyrotoxic effect of fipronil.

In majority of the cases, liver showed mild degenerative changes with diffuse necrosis, central venous congestion, sinusoidal congestion, hypertrophy and individualization of hepatocytes. There was an increase in AST level. These findings

indicated the hepatotoxicity of fipronil. Oxidative damage of the hepatocytes was evident, as there was an increase in lipid peroxides and decreased reduced glutathione and superoxide dismutase. The various histopathological lesions of liver observed were in accordance with the observations of various organizations and research workers (JMPR, 1997; Fluoride action network, 2004; WHO, 2007 and Wang *et al.,* 2008). Prominent kupffer cell reaction and basophillia of hepatocytes surrounding the area of damaged hepatocytes, central venous congestion, and sinusoidal congestion were the findings in group treated with curcumin. Some of the hepatocytes appeared bi nucleated in this group. Decreased serum AST Level and lipid Peroxides and increased reduced glutathione with superoxide dismutase in the liver observed in this experiment may be attributed to the hepatoprotection and antioxidant activity of the curcumin against the toxic effect of fipronil. Kumar *et al.* (2008) reported pretreatment of mice with curcumin resulted in hepatoprotection and reduced oxidative damage against paracetamol induced injury.

Degeneration and necrosis of the tubules and glomeruli, diffuse hemorrhages in the intertubular areas glomerular shrinkage, multifocal congestion and disorganized tubular epithelial cells were observed in rats which were treated with fipronil might be due to the fact that fipronil and most of its metabolites are excreted through kidney. These findings indicated the nephrotoxicity of fipronil. Anon (2004) observed progressive senile nephropathy in male rats when they were administered with fipronil. Mild degeneration of tubules and medullary haemorrhage were observed in group II. Most of the tubules and glomeruli appeared normal with usual cellularity. Mild damage of kidney could be attributed to protection carried by curcumin against toxic effect of fipronil on kidney. Cekmen *et al.* (2009) reported curcumin treated rats showed decreased renal lesions against acetaminophen induced nephrotoxicity.

Lung lesions varied from congestion, alveolar septal thickening, necrosis of alveolar cells, hemorrhage, oedema, peribronchial lymphoid cell hyperplasia and

bronchostenosis in group treated with fipronil. The lesions could be attributed to the toxic effect of fipronil on lung. Available literature does not show any information about overall toxicity of fipronil on lung, brain, heart, spleen, intestine and stomach. Meningeal congestion, predominance of red pulp with vacuolation of splenocytes, hyperkeratinisation of non glandular stomach, desquamation and fusion of villi and goblet cell hyperplasia in the intestine, vacuolization and hyalinization of muscle fibers and mild hemorrhage in the heart were observed in group treated with fipronil alone. Predominance of glial cells in the brain and white pulp predominance in the spleen, glandular hyperplasia of stomach and intestine, focal hemorrhage and congestion in heart were the major changes observed in group treated with fipronil and curcumin which indicated the stimulatory and protective properties of curcumin against toxic effect of fipronil on various organs. Group III (control) animals did not reveal any histopathological changes. Tingle *et al.* (2003) observed myocardial necrosis in dogs administered with 35 ppm fipronil desulfinyl.

From the present investigation it is clear that fipronil at 30.4mg/kg dose level for 28days is both thyrotoxic, hepatotoxic and nephrotoxic and better protection was seen offered by curcumin when it was incorporated along with fipronil proving that curcumin can be effectively used as an ameliorating agent against fipronil induced damage.

Summary

6. SUMMARY

The present experiment was undertaken to study the pathological effects of Fipronil and their amelioration by curcumin in rats.

Thirty adult male wistar albino rats weighing 150-200 g, divided into three groups comprising ten animals in each group, were used for the study. Group I animals were administered with fipronil at 30.4 mg/kg body weight/day in honey at 1.5ml/rat.Group II animals received both fipronil and curcumin at the dose of 30.4 mg/kg body weight/day and 100 mg/kg bodyweight respectively in honey at 1.5ml/rat. Group III animals were administered with honey at the rate of 1.5 ml/rat/day which were served as control. In all the experimental groups, oral administration was continued up to a period of 28 days and observed for symptoms.

Blood was collected on day 0, 7, 14, 21 and 28 for estimation of Hb, ESR, DLC, TLC and PCV and serum was used for the Total Protein, Albumin, Globulin, AST, ALT, Creatinine and Cholesterol. At the end of the experiment, all the animals were sacrificed and detailed postmortem examination was conducted and lesions were recorded. Weighed quantity of liver was collected in chilled normal saline for estimation of lipid peroxides, reduced glutathione and superoxide dismutase. Liver, kidney, brain, thyroid, spleen, heart, stomach and intestine were examined histopathologically.

Body weight of rats in all the groups showed a gradual increase throughout the experimental period. A significant decrease in the mean body weights was observed in group I and II compared with group III. Group II showed significantly higher mean body weights compared with group I. A significant reduction in mean total protein, albumin and globulin levels were observed in group I compared with group II and III. Group II showed significantly higher total protein, albumin and globulin mean levels compared to group I. AST and ALT levels showed significant increase in group I which indicated damage to the liver. Significantly lower AST and ALT levels was observed in group treated with curcumin which indicated hepatoprotective and antioxidant property of

curcumin against the pathological changes induced by fipronil. On 21st and 28th days, a significant increase in the cholesterol level was observed in the group I compared to group II and group III. The cholesterol level was found decreasing gradually from seventh day in group II but statistically significant decrease in cholesterol level was observed on days 21st and 28th compared to group I. On day 28th, group I showed significantly higher mean creatinine level compared to group II and group III. Group II showed significantly lower creatinine level compared to group I. The study of haematological parameters like TLC and DLC revealed that they were not adversely affected. On days 14th, 21st and 28th, group I showed significant decrease in the mean packed cell volume (PCV) compared to control group. Meanwhile significant decrease in the hemoglobin concentration (Hb) was observed in group I on day 28th compared to control group. The group II showed significant increase in the PCV and Hb values in these days which indicated that hepatoprotective property of curcumin. An increase in the levels of lipid peroxides and a concurrent decrease in reduced glutathione and superoxide dismutase in liver were observed in group I which indicated oxidative damage to the liver. Group II showed significant reduction of lipid peroxides and an increase in reduced glutathione and superoxide dismutase levels in the liver which indicated the protective effect of curcumin against oxidative damage caused by fipronil.

Two animals showed dullness, anorexia, abnormal gait, convulsion and aggressiveness in fipronil intoxicated group before their death. One in group II showed inappetence and depression before death and the other animals remained healthy and did not show any symptoms. Mortalities were observed in both group I and group II. Twenty percent mortalities were observed in the group I whereas group II showed 10 percent mortality only.

Gross pathological changes observed in group I animals were hepatomegaly, congestion, grayish white spots of necrosis in the liver, enlargement of thyroids, congestion and hemorrhage of the lung and kidney. The intensity of these gross lesions in group II were much less or almost absent compared with group treated with group I.

Group III did not reveal any macroscopical changes changes. On histological examination, thyroid revealed smaller acini with reduced amount of colloid, scalloping of colloid, hyperplasia, follicular cell hypertrophy, cystic dilatation of acini, fibrosis and desquamation of lining cells in group I. Uniform sized follicular acinus filled with colloid was observed in most of the group II animals. In majority of the cases liver showed mild degenerative changes with diffuse necrosis, central venous congestion, sinusoidal congestion, hypertrophy and individualization of hepatocytes in group treated with fipronil alone. Prominent kupffer cell reaction and basophillia of hepatocytes surrounding the area of damaged hepatocytes, central venous congestion and sinusoidal congestion were the findings in group treated with curcumin. Degeneration and necrosis of the tubules and glomeruli, diffuse hemorrhages in the intertubular areas, glomerular shrinkage, multifocal congestion and disorganized tubular epithelial cells were observed in rats which were treated with fipronil. Mild degeneration of tubules and medullary haemorrhage were observed in group II. Most of the tubules and glomeruli in this group appeared normal with usual cellularity. Lung lesions varied from congestion, alveolar septal thickening, necrosis of alveolar cells, hemorrhage, oedema, peribronchial lymphoid cell hyperplasia and bronchostenosis in group treated with fipronil. Meningeal congestion, predominance of red pulp with vacuolation of splenocytes, hyperkeratinisation of non glandular stomach, desquamation and fusion of villi and goblet cell hyperplasia in the intestine, vacuolization and hyalinization of muscle fibers and mild hemorrhage in the heart were observed in group treated with fipronil alone. Predominance of glial cells in the brain and white pulp predominance in the spleen, glandular hyperplasia of stomach and intestine, focal hemorrhage and congestion in the heart were the major changes observed in group treated with fipronil and curcumin. Group III did not reveal any histopathological changes.

From the present investigation it is clear that fipronil at 30.4mg/kg dose level for 28days is thyrotoxic, hepatotoxic and nephrotoxic. Further it is proved that that curcumin can be effectively used as an ameliorating agent against fipronil induced damage.

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STUDIES ON THE PATHOLOGICAL EFFECTS OF FIPRONIL AND THEIR AMELIORATION BY CURCUMIN IN RATS

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ABSTRACT

The present study entitled 'Pathological effects of Fipronil and their amelioration by curcumin in rats' was undertaken by administering with fipronil in group I animals and both fipronil and curcumin in group II animals for 28 days. Group III administered with honey served as control. The weekly body weights, clinical signs, haematology, biochemical parameters, mortality pattern, gross pathology and histopathology of various organs were analysed to study the effect. The oxidative damage of the liver was assessed by the estimation of lipid peroxides, reduced glutathione and superoxide dismutase.

A significant decrease in the mean body weights was observed in group I and II. ALT, AST, cholesterol and creatinine levels showed a significant increase in the group I and significantly lower levels in group II. Total protein, albumin, globulin, PCV and Hb levels were significantly lower in group I but significantly higher in group II. TLC, ESR and DLC revealed no variation. Group I showed significantly higher lipid peroxides and lower glutathione and superoxide dismutase levels in the liver. Group II showed significantly lower lipid peroxides and higher glutathione and superoxide dismutase levels in the liver.

The animals showed dullness and inappetance in the treatment groups. Mortalities were observed in both groups. Hepatomegaly and focal necrotic spots in the liver, enlargement of thyroid were the major gross lesions in group I. Gross lesions were less in group II. Smaller and cystic dilatation of acini, hyperplasia and fibrosis of thyroid, necrosis, hypertrophy and individualization of hepatocytes, tubular and glomerular degeneration and necrosis of the kidney, alveolar septal thickening, peribronchial lymphoid cell hyperplasia and bronchostenosis of the lung, predominance red pulp of spleen, desquamation and fusion of villi and goblet cell hyperplasia in the intestine, hyalinization of cardiac muscle fibers were observed in group I animal. Uniform sized follicular acini of thyroid, prominent kupffer cell reaction of hepatocytes,mild degeneration of tubules of kidney, predominance of white pulp of spleen, glial cell response in the brain, glandular hyperplasia of intestine were the major findings in group II animals. The study revealed that fipronil is thyrotoxic, hepatotoxic and nephrotoxic to rats and curcumin has good ameliorating effect against fipronil toxicity.