Patho-morphorphological and Clinico-pathological alteration of induced chloropyrifos toxicity in Vanraja birds

THESIS

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IN

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BY

Dr. Sikandar Yadav

Registration No – M/VPP/226/BVC/2014-15

(Veterinary Pathology)

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PATNA 800014

2017

DEPARTMENT OF VETERINARY PATHOLOGY
Bihar Veterinary College, Patna-800014.
(Bihar Agricultural University, Sabour, Bhagalpur, BIHAR)

CERTIFICATE-I

This is to certify that the thesis entitled **"Pathomorphorphological and Clinico-pathological alteration of induced chloropyrifos toxicity in Vanraja birds"** submitted in partial fulfillment of requirement for the degree of **Master of Veterinary Science (Veterinary Pathology)** of the faculty of post-Graduate Studies, Bihar Agricultural University, Sabour, Bhagalpur, Bihar is the bonafide research carried out by **Dr. Sikandar Yadav**, **Reg. No- M/VPP/226/BVC/ 2014-15,** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

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Date _____ (Sikandar Yadav)

Place _____


DEDICATED
TO
MY REVEREND PARENTS
TEACHERS

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Abbreviations

%	:	Percentage
AChE	:	Acetylcholinesterase
AST	:	Aspartate aminotransferase
ALP	:	Alkaline phosphatase
b.w.	:	body weight
CPF	:	Chlorpyrifos
conc.	:	Concentration
C	:	Control
cc	:	cubic centimeter
D	:	Day
DLC	:	Differential leukocyte Count
DCP	:	Digestible crude protein
DM	:	Dry Matter
DMI	:	Dry Matter intake
DW	:	Distilled water
ESR	:	Erythrocyte sedimentation rate

EDTA	:	Ethylene diamine tetra-acetic acid
EE	:	Ether extract
et al	:	et alibi
FAO	:	Food and Agriculture Organization
FCR	:	Feed Conversion Ratio
G	:	Globulin
GSH	:	Glutathion
g	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
IU	:	International Unit
Kcal	:	Kilo Calorie
Kg	:	Kilogram
LDH	:	Lactate dehydrogenase
L/N	:	Lymphocytes/Neutrophils
ME	:	Mechanical energy
mg	:	Milligram
ml	:	Milliliter
NRC	:	National Research Council

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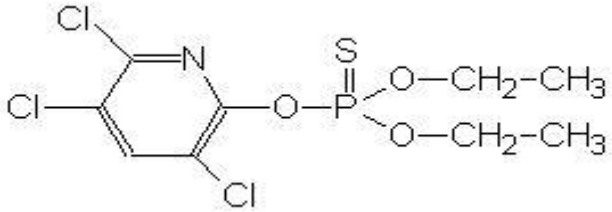
1 INTRODUCTION

Advent of agricultural and industrial revolution on global level has added many pollutants in the environment, which are potentially hazardous, out of which some may be toxic, inflammable, explosive or corrosive. Insecticides are one of the various pollutants present in the hydrosphere and lithosphere, which is toxic to livestock as well as human beings.

Due to their high insecticidal activity, low environmental persistence and moderate toxicity, the organophosphorus (OP) compounds are the most favored insecticides. They are widely used in agriculture and veterinary medicine. However, the unregulated use and its aerial application over large agricultural and urban areas have caused severe environmental pollution. Exposure to OP is associated with toxic effects on humans and animals **(Heikal et al., 2010, 2011, 2012; Goel et al., 2005;** Saulsbury, 2009**).**

OPs are the esters of pentavalent phosphorous acid. Among the several organophosphorus (OP) insecticides, the Chlorpyrifos [CPF: O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate], a broad spectrum insecticide has gained popularity in veterinary medicine globally.



Chlorpyrifos (chemical structure)

Chlorpyrifos (CPF) is a white crystalline solid with a strong mercaptan odour (Worthing, 1987). It does not mix well in water so it is mixed in oily liquids before application to crops or animals. It may be applied to crops in a micro-encapsulated form. CPF is the active ingredient of various commercial insecticides Organophosphates (OPs) including Dursban® and Lorsban® (ATSDR, USA, 1997).

Mechanism of Action

CPF induces neurotoxicity and tissue damage with observable signs of poisoning. CPF acts on the nervous system of the mammals, birds, fish and many organisms. It acts as acetylcholinesterase (AChE) inhibitor, an enzyme that hydrolyzes acetylcholine (ACh) as OPs bind irreversibly to AChE (Eaton *et al*., 2008). ACh is involved in the transmission of nervous signals from neuromuscular junctions and cholinergic brain synapses. It is one of the molecular mechanisms that have been implicated in CPF induced toxicity (Gultekin *et al*., 2001; Ambali *et al*., 2010). Also CPF causes deleterious effects through AChE inhibition at synapse of central and peripheral nervous system (Gordon *et al*., 1997). Prolonged exposure to CPF has been shown to cause anaemia (Ambali, 2009) and severe damage to the vital organs.

However, not all the pesticides are actually toxic for humans or other non-target species (U.S.P.H.S., 1995). In veterinary practice some pesticides are most commonly used as acaricides or ectoparasiticides for pest control in animals and birds. OP insecticides exhibit a wide range of toxicity in mammals (Sultatos, 1994). It is

used as an insecticide for grain, cotton, field, fruit and vegetable crops as well as on lawns and ornamental plants as premise insecticide including chicken houses because of its efficacy against a variety of pests, mites, lices and flies affecting the livestock and poultry (Strickland *et al*., 1970; Loomi *et al*., 1972; Leidy *et al*., 1991; Blagburn and Lindsay, 2001).

The indiscriminate use of insecticides has led to a widespread concern over the potential adverse effects of these chemicals on human and animal health. Accidental or careless applications of organo-phosphorus compound have resulted in the death of many species of non target organisms such as fish, aquatic invertebrate, birds and humans. General agricultural uses of CPF pose a serious hazard to life (Kidd and James, 1991). The exposure to low levels of CPF over a longer period would have more serious impacts on human and animal health. Unfortunately, the biological activity of CPF is not limited just to insects but also toxic to mammals and other non-target organism in which the cholinesterase enzyme plays a vital role.

CPF is readily absorbed into the blood stream through the gastrointestinal tract(GIT) if it is ingested, inhaled through the lungs or contact through the skin on dermal exposure (U.S. Public Health Service, 1995). There are reports of chronic CPF toxicity to birds, which showed adverse effects on fertility, hatchability and embryo deformities including twisted necks and shortened/ indented backs in bobwhites and adult chickens (Schom *et al*.,1973) and reduction in

body weight, egg production, eggshell thickness, egg weight and hatchling weight (Gile and Meyers, 1986).

CPF has also been reported to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress thereby disrupting the normal cellular development and differentiation. Low level of chronic exposure to agricultural chemicals may not have clinically recognizable symptoms but could produce subtle cumulative effects that eventually affect the health of organism (Bebe and Panemanglore, 2003).

Due to presence of residue of CPF in the soil, water, forages of ecosystem there is possibility of health hazards to the farm animals, poultry, human beings and wild animals. However, scanty reports are available on the clinicopathological implications of CPF. Therefore, the present study was conducted to investigate the chronic clinic-pathological implications of CPF in Vanraja birds.

Keeping in view the above mentioned points, the present study was undertaken with the following **Objectives**:

1. To study the body weight and clinical signs.
2. To study the haematological parameters.
3. To study the biochemical parameter.
4. To study the histopathological changes of visceral organs.

2 REVIEW OF LITERATURE

Globally pesticide application is increasing, particularly in the third world countries (AL-Badrany *et al*., 2007) including the control of termites in chicken houses (Leidy *et al*., 1991). Chemical pesticides cause health consequences to the birds culminating in great economic loss. It is also posing a potential threat to public health due to the presence of pesticide residues in poultry meat and egg (Begum *et al*., 2015). Toxicological studies of CPF in chickens focused on the sub-acute effects on plasma or serum enzymes and other biochemical parameters (Malik *et al*., 2004), examination of delayed neurotoxicity (Richardson *et al*., 2004) developmental effects (Geller *et al*.,1998) and pathology of long-term exposure (Krishnamoorthy *et al*.,2007).

Work done in India and abroad

CHLORPYRIFOS TOXICITY IN GENERAL

Human and animals are occasionally and unintentionally exposed to lethal and sub-lethal doses of pesticides (Eddlestone, 2000 and Martin *et al*., 2003). Human and animals can be directly

exposed to pesticides by inhalation, ingestion, contact with skin and eyes. Apart from the direct exposure, indirect exposure occurs in animals by consuming prey that contains high residues of the pesticides.

Schom *et al*., (1973) reported about chronic toxicity of chlorpyrifos to birds, having adverse effects on fertility, hatchability and embryo deformities- twisted necks and shortened/indented backs in bobwhites and adult chickens.

Nancy *et al*., (1994) reported various sublethal effects of pesticides organochlorines and OPs in animals abnormal ovulation and egg-shell formation in chicken, reproductive and developmental toxicity and endocrine disruption. These OPs (e.g. chlorpyrifos and methidathion) with a very narrow safety margin in birds accumulate in their system to their detriment. Such Birds exhibit muscular incoordination, salivation and vomition, diarrheal, unconsciousness and death. CPF elicits a number of other effects including hepatic dysfunction, immunological abnormalities, embryotoxicity, genotoxicity, teratogenicity, neurochemical and neuro-behavioral changes.

Gayathri *et al*. (1998) stated that the effects are obvious soon after exposure to lethal dose of pesticides leading to high economic losses in animals and death in extreme cases.

Blakley *et al*., (1999) reported that the exposure of laboratory animals particularly to CPF elicits immunological abnormalities.

Gomes *et al*., (1999) reported that the widespread use of OP insecticides particularly CPF has long been shown to exert deleterious effects including hepatic dysfunction on living organisms.

Bolognesi and Morasso, (2000) reported that human and animal populations globally are exposed on daily basis to low levels of environ-mental contaminants. Pesticides such as OPs are one of the most important environmental ontaminants as they remain inevitably present as residues in food from both vegetal and animal origins.

Mohammad *et al*., (2008) reported that on acute (24 h) oral LD_{50} of CPF in chicks 10.79 mg/kg, the signs of cholinergic toxicosis appeared within two hours after dosing and they included salivation, lacrimation, gasping, frequent defecation, drooping of wings, tremors, convulsions, and recumbency before death. With half the LD_{50} dose of CPF (5 mg/kg), it caused immobility and wing drooping. OPs reduced plasma and whole brain ChE activities by (29 to 84) % and (18 to 77) %, respectively, depending on the dose applied. Correlation analysis showed that the inhibition of brain ChE activity correlated well (r=0.82) with that of the plasma ChE activity.

Gulati *et al*., (2015) reported that CPF intoxication produces hematological, biochemical, and pathological changes in treated birds. Cholesterol level was only significantly reduced in the plasma, Plasma glucose values significantly increased. Microscopic lesions in various organs of broiler chickens indicating cellular toxicity in these organs alongwith the immunotoxic effects of chlorpyrifos on

avian lymphocytes i.e. apoptotic death in lymphocytes (Ravindra et al., 2004).

Begum *et al*., (2015) reported that chronic CPF intoxication in birds produced hematological, biochemical and pathological changes. A significant ($P<0.01$) increase of Hb, TEC, TLC and hetrophil percent and decrease of lymphocyte percent was found. Serum ALP, AST, ALT and uric acid increased significantly. The protein level remained similar.

CLINICAL SIGNS

Choloropyrifos intoxication shows various clinical signs.

Gile and Meyers (1986) reported reduction in body weight, egg production, eggshell thickness, egg weight and hatchling weight. Kamrin (1997) reported that the signs of CPF intoxication in birds include excessive blinking, hypoactivity, excitability, excessive drinking, salivation, diarrhoea, lacrimation, muscular in coordination, muscular weakness, tremors, rapid breathing and fluffed feathers.

Malik *et al*., (2004) reported that in CPF treated birds a non-significant decrease in body weight and feed efficiency was observed.

Ramish (2007) reported depression, reduced feed intake and dullness as clinical symptoms accompanying exposure of birds to sub-lethal dose of a pesticide. There was no alteration in haematological

parameters but a significant dose dependent increase in serum enzymes was observed.

Slotkin *et al*., (2008) reported that CPF exposure reduced the hatch rate and increased the incidence of birth defects. There were no significant CPF effects on body or brain region weights of the newly-hatched chicks.

Kammon *et al*. (2010) studied the effect of CPF in the layers by administering @ of 55 mg/kg body weight orally. The CPF produced signs of toxicity commencing two hours after administration, which included excitation followed by sluggishness, watery diarrhea, excessive salivation, changing to drooling and rigid stance with dropping of wings. The chickens were unable to stand and showed convulsions before death.

Ahmad *et al*., (2015) reported thar birds exposed to high dose (20mg/kg BW) showed signs of toxicity- salivation, lacrimation, gasping, convulsions, frequent defecation and tremors. The birds exposed to 10 and 20mg/kg showed significantly ($P \leq 0.05$) decreased body weight.

Wani (2015) reported that the CPF treated birds resulted in a significant decrease in feed consumption, body weight, body weight gain and increase in FCR with that of control group birds in dose and duration dependent manner. Clinical signs observed upto 3 weeks were less prominent in all the treatments. However, after 4 weeks PE, the birds of treatment group showed varying degree of toxic signs

which could be supported by the adverse effects of the chlorpyrifos toxicity.

HAEMATOLOGY

The acute CPF intoxication produce changes in hematology of the treated birds.

Ambali *et al.* (2010) evaluated the effect of acute chlorpyrifos exposure on short-term hematological changes in Wistar rats, and the ameliorative effect of vitamin C. CPF group showed a 10% drop in Hb concentration compared to 2.1% decrease recorded in the Vit. C+CPF group. The RBC count in the CPF group was lowered by 11%, compared to those recorded in the Vit. C+CPF (2.6% drop). Rats in the CPF group had a 7.8% increase in WBC concentration. On the other hand, the Vit. C+CPF group had a 14.1% decrease in WBC concentration

Kammon *et al.*, (2011) reported that chronic exposure of broilers to chlorpyrifos at 0.8 mg/kg bw has no significant toxic effects on haemopoietic system i.e the CPF administration at 0.8 mg/kg bw did not produce any significant changes in the concentration of haemoglobin, TLC and DLC in broiler chickens on day 24 and day 45.

Ahmad *et al*., (2015) reported that there was significant (P≤0.05) decrease in hematological parameters i.e. total erythrocyte counts, hemoglobin concentration, hematocrit and total leukocyte in the high dosed group as compared to control and other low dosed fed birds.

Begum *et al*., (2015) reported that the Hb, TEC, and TLC concentration was significantly (P<0.05) increased from 6 h onward in treated as compared to control chickens. The lymphocyte percent showed decreasing trend while the heterophil percent showed increasing trend in treated than the control group.

BIOCHEMICAL STUDIES

The acute CPF intoxication produce changes in biochemical constituents of the treated birds.

Malik *et al*., (2004) reported that CPF even in low dosages in the diet has deleterious effects on chicken body metabolism. The blood glucose and total plasma proteins showed a significant (P<0.05) increase at all doses in proportion to the dose level. CPF exposure produced hypercholesterolemia, which was significant from third week onwards in all the treated groups. Increased serum uric acid level was observed in all test groups. The serum electrolytes concentration revealed an increase in the mean serum sodium level and a decrease in mean potassium level compared to control.

Kumar *et al*., (2007) studied the effect of CPF on the fresh water field crab, which revealed biochemical changes in the

neurosecretory cells such as brain, thoracic ganglia and eyestalk. There were changes in enzymatic assay such as lactate hydrogenase (LDH), succinate dehydrogenase (SDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) in the neuro-secretory cells.

Mohammed *et al*., (1990) reported an increase in total serum protein in goats induced with acute chlorpyrifos and chlorpyrifos-methyl 1:1 ratio mixture @ 300 mg/kg body weight when given to goats resulted in decrease in total serum protein.

Malik *et al*., (2004) repoted that the continuous presence of CPF even in low dosages in the diet has deleterious effects on chicken body metabolism. The blood glucose and total plasma proteins showed a significant ($P<0.05$) increase at all doses in proportion to the dose level. CPF exposure produced hyper-cholesterolemia, which was significant from third week onwards in all the treated groups. Serum uric aid level increase was observed in all test groups. An increase in the mean serum sodium level and a decrease in mean potassium level compared to control.

Ojezele and Abatan (2009) reported the toxicological effects of the chlorpyrifos and the usefulness of some parameters as bioindicators. Serum biochemistry was assayed in 15 cockrels at random age of 4-6 weeks. The birds were randomly assigned to 3 oral (by mouth) pulse-dose treatments of 0, 3.5 mg/kg CPF. CPF caused significant decrease in the levels of ALP, ALT, TP, and albumin while significant increase in the level of AST.

Bharathi *et al*., (2011) reported concentrations of the total cholesterol, LDL and triglycerides were significantly increased in toxic control with a significant decrease ($P<0.05$) in HDL concentration. The study revealed a significant ($P<0.05$) decrease in the concentrations of total protein, albumin and globulin (g/dl) and the A/G ratio in CPF toxic control group at the end of 4^{th} week.

Kammon *et al*., (2011) reported unaltered serum AChE activity, serum AST and ALT were slightly elevated in CPF-treated group. AST serum activity increased with age. CPF did not significantly influence the serum AKP activity, but this enzyme was slightly elevated in CPF-treated group on day 45 of age. AKP serum activity was higher on day 24 as compared to its activity on day 45. CPF did not produce significant changes in serum levels of glucose, cholesterol, creatinine, total protein, albumin, uric acid and activity of CK.

Ahmad *et al*., (2015) reported biochemical alteration in high dosed CPF fed birds. In Porotein profiling, Serum protein and albumin showed a significant ($P\leq0.05$) increase while non significant results in the case of globulin. The acetylcholinestrease (AChE) activity was significantly ($P\leq0.05$) decreased in blood, serum and plasma in CPF fed birds. A significant ($P\leq0.05$) higher levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was found.

Begum *et al*., (2015) reported biochemical alterations due to induced CPF toxicity between treated and control groups. The mean ALP, AST, and ALT activities increased significantly ($P<0.05$) from 6 h

onward till the end of the experiment, mean values of CHE activity showed significant ($P<0.05$) inhibition, the total protein showed nonsignificant variation and mean serum uric acid significant ($P<0.05$) increase from 6 h to 36 h.

Histopathology

Chlorpyriphos is known to produce pathological lesions in multiorgan systems.

Malik *et al*., (2002) reported that CPF fed broilers @ 30, 60 and 120 ppm from 0 to 6 weeks of age revealed hepatocellular necrosis, desquamation of kidney tubular epithelium, degeneration of myocardium and a few neurons and Purkinje cells in brain.

Yadav *et al*., (2003) reported that CPF fed broilers @ 35, 70 and 140 ppm of CPF from 2 to 8 weeks of age showed congestion and haemorrhages of liver, lung, intestine and thigh muscles.

Krishnamoorthy *et al*., (2007) studied the effect of CPF on the organs. **The lesions observed in liver were periportal fibrosis, bile duct hyperplasia and focal mononuclear cell collection.** Chicks were fed CPF @45 mg/kg for 28 days from day of hatching. The study of lymphoid organs- bursa of Fabricius, caecal tonsils and spleen showed various changes. The bursa of Fabricius showed lymphoid depletion and necrosis in less than 50% of cells and mild interfollicular fibrosis. Caecal tonsils revealed necrosis and depletion of lymphoid cells at 14^{th} and 28^{th} day. At 14^{th} day spleen showed depletion and necrosis in less than 50% of lymphoid cells with mild

reticulum cell hyperplasia and at 28th day of study depletion and necrosis in more than 50% of lymphoid cells with marked reticulum cell hyperplasia were observed. Harderian gland of birds showed depletion of plasma cells and necrosis of the gland on 14th and 28th day of age.

Tripathi and Srivastav (2010) reported the histopathological changes in liver of rats caused by chlorpyrifos administration @ 5 mg/kg and 10 mg/kg body weight daily up to 8 weeks. The histopathological changes in liver of rats by chlorpyrifos administration were mainly hepatocytic vacuolation, degeneration of hepatocytes and their nuclei, hyperchromatic and hypertrophied nuclei, sinusoidal dilation and focal necrosis at earlier stage of treatment.

Sodhi et al. (2008) investigated the role of a tocopherol and selenium on malathion induced hepatic damage, and antioxidant defense in chicks. The chicks were divided into three groups. First group received malathion 10 mg/kg body weight, orally for 60 days. The second group was administered with the same dose of malathion but supplemented with a tocopherol and selenium for 60 days. The third group served as control. Histopathological studies of liver in the chicks which received malathion exhibited, moderate to severe degenerative and necrotic changes in the hepatocytes. The correlation of decreased antioxidant status of chicks with degenerative changes in liver suggested that lipid peroxidation might be one of the important mechanism in the chronic toxicity of malathion. The results indicated

that a tocopherol and selenium were effective in partially alleviating degenerative changes induced by malathion in the liver of chicks by attenuating processes leading to lipid peroxidation.

Kammon *et al*., (2010) reported that in CPF induced toxicity in layers, the histopathological examination of liver tissues showed degeneration (cloudy swelling with mild fatty changes), coagulative necrosis and hemorrhages. The kidneys showed hemorrhages, vacuolar degeneration of tubular epithelial cells besides coagulative necrosis.

Bharathi *et al*., (2011) reported hepatic damage in histological sections of liver in CPF toxic control group, which showed marked central vein congestion, hydropic degeneration, mild bile duct hyperplasia and dilated sinusoidal spaces with congestion in the sinusoidal spaces.

Kammon *et al*., (2011) reported histopathological variations in different organs of CPF fed birds. Proventriculus showed degenerative changes in the mucosal papillae tips, necrosis of glandular cells and accumulation of exfoliated cells in the glandular lumen. Intestine showed increased number of goblet cells (mucous degeneration) and necrosis associated with sloughing of the epithelial cells. In Liver, dilation of hepatic sinusoids, vacuolar degeneration and fatty changes, coagulative necrosis and proliferation of bile duct cells was found. Pancreas showed degeneration and necrosis of glandular acini, degeneration and

necrosis of beta cells and proliferation of interlobular ducts. In Kidney, it was congestion and haemorrhage, vacuolar degeneration of tubular epithelial cells, coagulative necrosis, sloughing of tubular cells, necrosis of glomeruli and proliferative glomerulitis. Brain showed swelling of capillary endothelial cells, vacuolization of neurons, neuronal degeneration and necrosis, congestion and haemorrhages in cerebellum and degeneration of Purkinje cells. Heart showed granular degeneration and infiltration of inflammatory cells.

Roopadevi *et al*., (2012) reported that CPF inoculated chick embryos showed grossly hemorrhages on the head and in thigh region, liver showed congestion and streaks of pale areas with distended gall bladder, kidney revealed congestion and hemorrhages in all the treatment groups alongwith tubular epithelial cells showed varied type of degeneration characterized by granular eosinophilic cytoplasm with vacuoles and occlusion of tubular lumen with eosinophilic debris. Heart showed congestion, oedema and infiltration of inflammatory cells between the cardiac muscle fibres, cardiac myocytes exhibited swelling and mild vacuolar change with granular cytoplasm. Such lesions were recorded in broiler chickens due to CPF toxicity. Proventriculus showed degeneration and desquamation of mucosal epithelial cells along with infiltration of mononuclear cells into lamina propria.

Ahmad *et al.,* (2015) reported necrotic and degenerative changes on histopathological investigations of spleen, kidneys, bursa of Fabricius, thymus and brain tissues in CPF exposed birds.

3 MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Design of study

The study was designed for pathomorphological and clinicpathological alterations of Chloropyrifos (CPF) induced toxicity in Vanraja birds.

3.1.2. Area of study

The study was conducted in Vanraja birds reared at Institutional Livestock Farm Complex (ILFC), Bihar Veterinary College, Patna – 14.

3.1.3. Sample collection

Birds were reared for biochemical, haematological and histological examination for which blood samples were collected. The different types of samples used in the present study are given in Table 1.

Table A . Sample collected from Vanraja/grampriya birds:

S. No.	Group	Types of Samples	No. of samples

1.	Gr I	Blood	EDTA	4
			Non EDTA	4
		Histopathological	4	
2.	Gr II	Blood	EDTA	4
			Non EDTA	4
		Histopathological	4	
3.	Gr III	Blood	EDTA	4
			Non EDTA	4
		Histopathological	4	
4.	Gr IV	Blood	EDTA	4
			Non EDTA	4
		Histopathological	4	
	Total		48	

3.1.4. Chemicals used for studies

All the chemicals used in the study were procured from Excel crop care private limited, Mumbai. Chemicals used in the study included Chloropyrifos (20%), 0.1N hydrochloric acid (0.1 N HCL), Leismann stain(1:9 dilution).

3.1.6. Plasticwares and glasswares

Plasticwares used in this study were procured from Tarsons (India), Hi Media (India), and Axygen (USA) whereas glasswares were procured from Tarsons (India), Borosil (India) and Schott Duran (Germany). Glasswares were thoroughly washed and sterilized wherever necessary following the recommended procedures.

3.1.7. Equipments

Some important equipments used in the study were Sahli's haemometer, Deep Freeze (-20ºC) (Blue Star, India), Centrifuge,

Electronic Balance (Denver, USA), Microscope (Olympus, India), micropipette (Eppendorf, Germany) and Microtome.

3.2. METHODS

3.2.1. Experimental birds and their management

Day old Vanraja chicks (140 Nos) weighing about 40-50 gm were obtained from ILFC hatchery. Birds were housed in standard hygienic and managemental condition throughout the experimental period. All the unsexed birds were maintained in heated (35^0C) metal batteries up to 2 weeks, with chick mash feed and water *ad libitum,* for acclimatization. Then random division of birds into 4 equal groups of 35 each and shifted to non-heated cage with temperature 29^0C±3^0C, to determine the toxicopathological effects of CPF feeding. Vaccination done at 4 days and 28 days with RD-F_1 strain and booster respectively.

3.2.2. Chloropyrifos Treatment

Chlorpyrifos @ 35mg, 70 mg, 140 mg per kg feed in treatment groups I, II, III respectively while IV control group left untreated (Al-Badrany and Mohammad, 2007), each group consisted of 35 birds from 0^{th}day to 28^{th} day, fed orally using pulse chunni as vehicle. Control group was provided standard feed and plane water only.

Table B. Feeding of birds

Test Group	Feed/ kg feed	Route
Group I	Chlorpyrifos @35mg/ kg feed	Orally
Group II	Chlorpyrifos @70mg/ kg feed	Orally
Group III	Chlorpyrifos @140mg/ kg feed	Orally
Group IV	Normal feed and water	Orally

3.2.3. Clinical signs

All the experimental birds (Gr I, II, III & IV) were kept under strict supervision for whole trial period. The birds during experiments were examined daily twice and progressive developments of clinical signs of toxicity including mortality (if any).

3.2.4. Body Weight

The initial & weekly body weight were analyzed for the same periods to measure the effect of chlorpyrifos toxicity.

3.2.5. Hematological studies

Blood sample (3 ml) were collected from 5 birds randomly, in a BD Vacutainer K2 EDTA 5.4 mg at the 0, 3, 7, 14, 21, 28th day of age from the wing vein of birds of all the groups (I, II, III and IV) using sterile disposal syringe. Blood taken for hematological parameters i.e. Hemoglobin (Hb), Packed cell Volume (PCV), were estimated by the method of described by Jain (1986). Total Erythrocyte count (TEC) & total Leukocyte count (TLC) were assessed by the method of Natt

and Harrik (1952). Differential Leukocyte count (DLC) was done by preparing a thin blood smear from a drop of blood. Smear was air dried fixed in methanol for 2-3 minute and stained with 1:10 dilution Giemsa stain for 30 minute (Lucas and Jamroz, 1961).

3.2.6. Biochemical assay

Blood sample (4 ml) were collected from wing vein of all the groups at 0, 3, 7, 14, 21, 28th day of age in a BD Vacutainer serum tube without any anticoagulant and was allowed to clot. The separated serum was utilized for estimation of tatal protein and albumin, serum creatinine, uric acid, aspartate aminotransferase(AST), alanine aminotransferase (ALT) was done by using Coral, Span and Rapid H dignostic kits.

3.2.7. Gross pathology

Birds of all the groups were sacrificed by cervical dislocation at the end of 4 weeks and gross pathological lesions were recorded immediately after slaughter. Different organ were collected in 10% formalin for histopathological processing.

3.2.8. Histopathology

Tissue pieces of liver, kidney, brain, lungs, heart and intestine were preserved in 10% formalin solution immediately after necropsy. After proper fixation in formalin for 3-4 days, the tissues were cut into block of 2 cm^2 and placed under running tap water in order to remove formalin from tissues. The tissues were dehydrated in order-

50%>70%>95%>absolute alcohol for 1h in each. The tissues were then kept in cedar wood oil until cleared. Finally, the tissues were placed in melted paraffin (congealing point 58^0-60^0C) from No.1 to No.4 gradually for 1h in each and blocks were prepared by embedding tissues in fresh melted paraffin.

Tissue sections of about 3-5 µ thick were cut in a microtome. Sections were stained with Mayer's haematoxylin and eosin (Luna, 1968), for histopathological examinations (Bancroft and Stevens, 1980). Finally, the sections were mounted over a grease free glass slide with help of cannada balsam covered by a glass cover and examined under microscope.

3.2.9. Statistical analysis

All the estimated data was analyzed by analysis of variance and the significant results were shown by difference in superscripts with the respective means (Snedecor and Cochran, 1989).

4 RESULT AND DISCUSSION

Organophosphate (OP) insecticides are one of the most widely used chemicals in agriculture and public health (Ambali et al., 2010). Chlorpyrifos (CPF), an Opinsecticide, induces neurotoxicity and tissue damage with observable signs of poisoning. The primary mechanism of toxicity is associated with its ability and especially that of its metabolite, CPF inhibits acetylcholinesterase (AChE), an enzyme that normally terminates neurotransmission at cholinergic synapses (Eaton et al., 2008). Prolonged exposure to CPF has been shown to cause severe damage to the vital organs. In the present study, we investigated the chlorpyrifos-evoked haematological, biochemical and related histopathological alteration in Vanraja birds upon 2 weeks and 4 weeks post exposure have been correlated with the different dilutions of LD_{50} of chlorpyrifos administered.

The focus of attention in the present study was to evaluate the potentials of livestock, their handlers, agricultural environment for determine the toxicity of organophosphates. Primarily this chemical insecticide was prioritized because of the emergence of clinic-pathological and pathomorphological changes in CPF intoxicated vanraja birds. The clinicpathological studies included toxic signs and determinations of weekly body weight, haematological and biochemical alterations in serum as a result of indiscriminate use of in veterinary as well as human medicine which is a major public health concern.

4.1.Clinicopathology

4.1.1 Live body weight

The live body weight of birds of each group was recorded at weekly interval and expressed in grams (Table - 1). Since the chicks are in growing stage, all gained weights as progression of experiment.

The birds show decrease in body weight during trial but it was insignificant. Body weights of CPF induced broiler chickens GrI, II, III were significantly ($P<0.05$) lowered from 3 weeks onwards when compared with the control chickens (Table, Fig.). No significant change in the body weight of the birds was observed in between the GrI, II, III at different time intervals.

The growth depression effects persisted throughout the experimental periods. CPF poisoning caused severe diarrhoea causing

fluid loss, inappetance and a gradual loss of general condition. Reduced appetite, frequent diarrhoea along with the damage of liver, kidney and gastro-intestinal tract due to effect of CPF might have played an important role in body weight loss in toxicity. There was approximately 7.66% body weight loss in CPF induced GrI birds while 8.25% body weight loss in Gr II at 4 weeks post treatment than the control birds of that age whereas that in Gr III was 10.19%. The results clearly indicated that CPF had growth depressing effect which is in accordance with the reports of Kumar, (2011) in broiler birds. The adverse effect on the body weight gain may be due to CPF, which reduced to body tissue mass possibly via deteriorative changes in the fat and protein metabolism (Rowlands and Downey 2000). The reduction in body weight of the birds of the group Gr III were less as compared to the birds of Gr II. Wani *et al*. (2015), also found a significant decrease in body weight and body weight gain in the chlorpyrifos treated birds.

Table 1 : Body weight (g) in control and CPF treated Vanaraja birds

Age in Week (s)	Average body weight (g) of Vanaraja birds (n=20)				Percentage of reduction in growth in birds of Gr. I (%)	Percentage of reduction in growth in birds of Gr. II (%)	Percentage of reduction in growth in birds of Gr. III (%)
	Gr. I	Gr. II	Gr. III	Control Gr. IV			
1	166 ±2.98^{a}	160±3.04^{b}	155±3.514^{c}	176±2.92^{a}	5.68	9.09	12.07
2	280±4.14^{b}	276±4.62^{b}	261±4.85^{c}	292±3.28^{a}	4.10	5.47	10.61
3	622±5.16^{b}	610±5.41^{b}	590±4.61^{c}	659±4.80^{a}	5.61	7.43	10.47
4	951±8.04^{b}	945±7.72^{b}	925±6.62^{c}	1030±5.18^{a}	7.66	8.25	10.19

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

4.1.2 Clinical signs

Birds of Gr I did not show marked sign and symptoms except mild darrhoea and slight depression in some birds. Absence of nervous symptoms.

Birds of Gr II showed depression and unthriftness. Feed intake and water consumption of birds were slightly decreased. These symptoms arose after 3rd week post treatment.

Birds of Gr III exhibited diarrhoea and depression, unthriftness, droopiness, drowsiness, loss of appetite and respiratory distress. Feed consumption and water intake of birds were apparently decreased, with some showing excitement, salivation, incoordination, and respiratory distress in advanced stages. Hyper excitability, ataxia and sideways falling observed in birds after 4th week post treatment.

Birds of Gr IV (control) were in healthy condition as they were on normal diet.

During the course of experiment 3 birds in Gr I, 4 birds in Gr II, 7 birds in Gr III were died.

Mohammad *et al*. (2008) also reported similar clinical signs in chicks induced with OP compound respectively. Kammon *et al*. (2010) also observed similar clinical signs in CPF induced layer birds. The observed clinical signs in CPF treated birds in the present study

corroborated with the findings of Kumar (2011). OPs compound generally elicit their effect through the inhibition of Acetyl cholinesterase (AChE) leading to accumulation of this neurotransmitter in synapses with over stimulation of postsynaptic cholinergic receptors and consequent signs of neurotoxicity (Mileson *et al*., 1990; Monnet *et al*., 2000 and Ricceri *et al*., 2006).

4.2. Haematological studies

4.2.1 Haemoglobin

The mean weekly concentration of Hb (g/dl) is given in (Table - 2 and Fig – 1). Result showed an increase in the haemoglobin values There was significant increase ($P<0.05$) at 1st and 2nd week in all treatment level. However the difference between Hb level was found non- significant.

4.2.2 Packed cell volume

Weekly mean values of PCV were calculated as percentage and shown in (Table -2) Increase in PCV values were correspondence to the dose but the difference was non-significant among all treatment groups at different intervals.

4.2.3 Total Erythrocyte count

In Table – 2 shows total erythrocyte count in Vanraja birds raised an feed containing chlorpyrifos Result revealed an increase in TEC the increase was significantly higher ($P<0.05$) in group II and III than of group IV (control) at 4th week of chlorpyrifos treatment.

4.2.4 Differential Leucocyte count

The mean percentage values for DLC are presented in (Table – 3). Chiorpyrifos at different dose levels caused decrease in lymphocyte. The difference between various treatment group was significant at 1st, 2nd, 3 rd, and 4th week in all groups. The decrease was in proportion to the treatment level.

4.2.5 Total Leucocyte count

The mean value of TLC is presented in (Table 4 and Fig 2). The increase was found non- significant at 1st and 2nd week among the groups. At 3rd week, significant difference was found in treatment groups than in control group (Gr. IV). Birds of group I, II and III were differed ($P<0.05$) from group IV (control) birds at 4th week.

Results of hematological findings are summarized in table which showed a significant ($P<0.05$ or $P<0.01$) increase in Hb, PCV and TEC in CPF induced Vanraja birds compared to control birds after 2 and 4 weeks of treatment. Gr III birds showed significant improvement when compared to that of Gr II but the values were higher than that of the Gr IV.

These results also corroborated with the findings of Sastry, (1983), Malik *et al*. 2002, Begum et al. 2015, Obaineh and Matthew, 2009. The rise in Hb concentration, PCV and TEC in the present study might be due to the dehydration resulting in diarrhoea and excessive salivation.

Gr III birds showed significant improvement in the value of Hb (Table 2), PCV (Table 2) and TEC (Table 2) on 14 and 28 days of experiment clearly indicating the toxic effect of CPF.

In this study there is dose dependent decrease in the lympho-cytoneutrophil ratio in all the treated groups, indicating a relative decrease in lymphocyte count and increase in neutrophil count whereas the decrease in this ration was significant ($P<0.05$). The lymphopenia observed in the CPF intoxication may be due to either the decreased production and/or increased rate of removal due to rapid destruction. The pesticides are toxic to the cells of immune system through the induction of necrosis and apoptosis (Ambali *et al*., 2010).

Table 2: Hematological changes in control and CPF induced Vanaraja birds at 1st and 2nd week post treatment

Parameters	1st week				2nd week			
	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. I	Gr. II	Gr. III	Gr. IV
Hb (g%)	7.20± 0.28[a]	7.36± 0.32[a]	7.52± 0.34[a]	5.98± 0.47[b]	7.38± 0.28[a]	7.54± 0.30[a]	7.64± 0.54[a]	6.08± 0.37[b]
PCV (%)	26.02± 1.21	26.50± 1.27	26.60± 1.02	24.64± 1.06	26.52± 1.29	27.20± 0.98	28.70± 1.28	25.14± 1.28
TEC (10^3 /mm^3)	1.74± 0.18	2.05± 0.15	2.18± 0.22	1.67± 0.15	1.85± 0.13	2.10± 0.15	2.16± 0.20	1.75± 0.21

*****Means in a row having different superscripts differ significantly ($p<0.05$; $p<0.01$)

Table 2 : Haematological changes in control and chlorpyrifos induced Vanaraja birds at 3rd and 4th weeks post treatment

Parameters	3rd week				4th week			
	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. I	Gr. II	Gr. III	Gr. IV
Hb (g%)	7.48± 0.43	7.56 ±0.58	7.88± 0.48	6.12± 0.30	7.28± 0.42	7.60± 0.57	8.12± 0.30	6.48± 0.33
PCV (%)	27.66± 1.22	28.40± 1.07	29.50± 1.94	25.38± 1.02	28.20± 0.96	28.60± 1.57	29.84± 1.93	26.26± 1.14
TEC (10^3 /mm^3)	1.77± 0.18	2.24± 0.17	2.34± 0.18	1.78± 0.20	1.90± 0.26	2.26± 0.23	2.40± 0.30	1.84± 0.25

*Means in a row having different superscripts differ significantly (P<0.05)

Fig. 1 :Hb (g %) variation in control and CPF treated birds

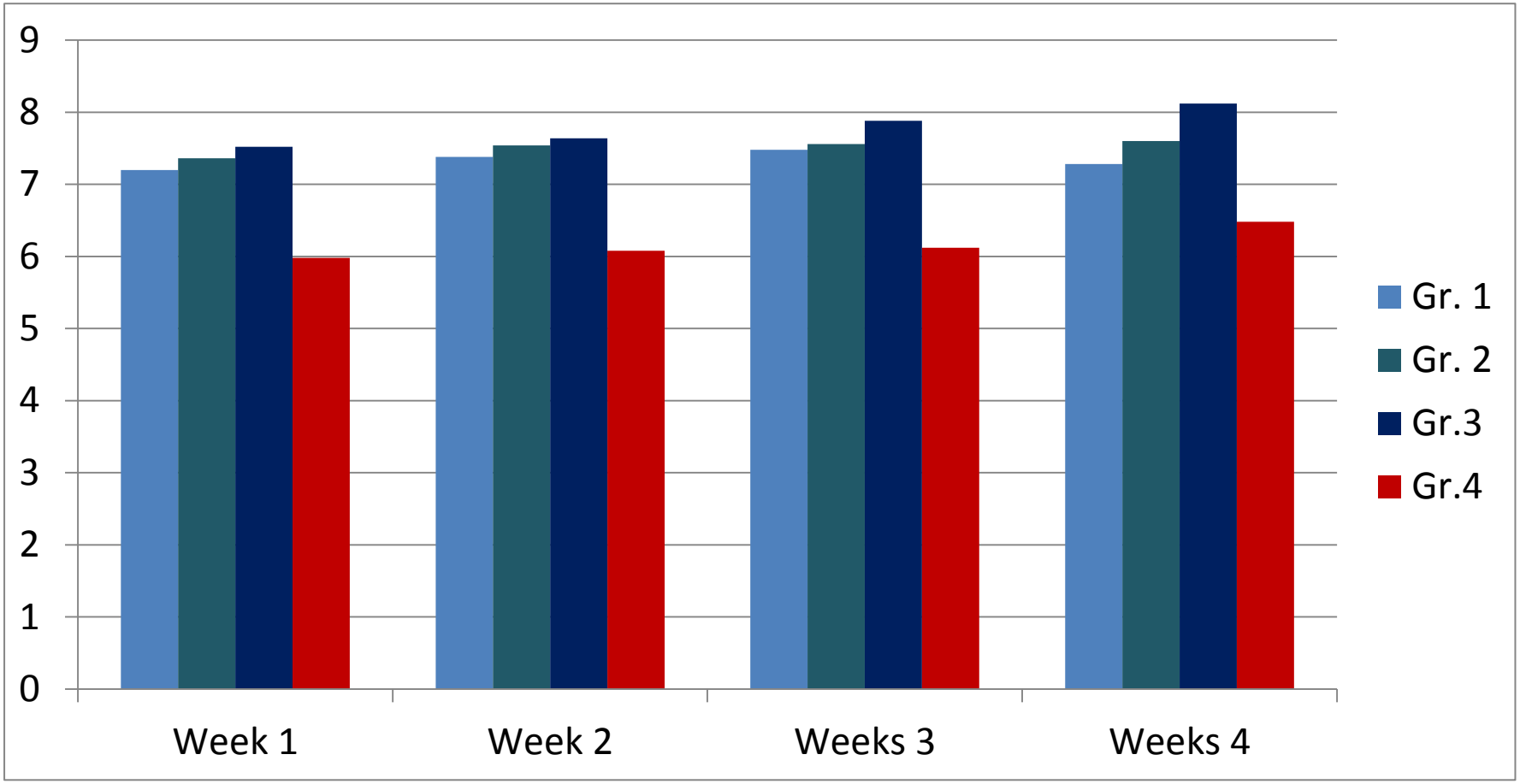


Table 3 : DLC of control and CPF treated birds

Parameters (%)	1st week				2nd week			
	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. I	Gr. II	Gr. III	Gr. IV
Lymphocytes (%)	60.54±1.80^{b}	60.20 ±1.71^{b}	58.50 ±1.67^{b}	66.94 ±1.84^{a}	59.6 ±1.69^{b}	57.10 ±1.38^{b}	56.40 ±1.20^{b}	66.50 ±1.61^{a}
Heterophils (%)	29.20 ±1.50^{a}	30.50 ±1.24^{a}	33.20 ±1.40^{a}	23.58 ±1.63^{b}	28.60±1.63^{b}	32.20±1.40^{a}	33.50 ±1.28^{a}	25.20±1.65^{c}
Monocytes (%)	6.10 ±0.45	4.80 ±0.35	5.10 ±0.39	5.84 ±0.22	6.12 ±0.40	5.50 ±0.25	6.0 ±0.33	4.40 ±0.20
Eosinophils (%)	3.0 ±0.57	3.40 ±0.48	2.40 ±0.53	2.64 ±035	4.50 ±0.42	4.30 ±0.27	3.28 ±0.32	2.85 ±0.35
Basophils (%)	1.16 ±0.26	1.10 ±0.28	0.80 ±0.14	1.20 ±0.25	1.20 ±0.24	0.90 ±0.18	0.82 ±0.16	1.05 ±0.28

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Table 3 : DLC of control and CPF treated birds

Parameters (%)	3rd week				4th week			
	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. I	Gr. II	Gr. III	Gr. IV
Lymphocytes (%)	59.80±1.40^{b}	58.50±1.40^{b}	56.80 ±1.05^{b}	65.20 ±1.39^{a}	61.40 ±1.65^{b}	60.50±1.60^{b}	56.40 ±1.32^{c}	67.20 ±1.19^{a}
Heterophils (%)	29.60 ±1.16^{b}	32.30 ±1.37^{ab}	33.20 ±1.10^{a}	24.60 ±1.07^{c}	28.20 ±1.61^{b}	31.20±1.13^{b}	35.20 ±1.47^{a}	25.30 ±1.13^{c}
Monocytes (%)	6.40 ±0.29	5.40 ±0.37	6.30 ±0.23	6.20 ±0.36	5.30 ±0.27	5.0 ±0.26	4.60 ±0.36	4.40 ±0.20
Eisonophiles (%)	3.60 ±0.37	3.10 ±0.29	2.92 ±0.28	3.12 ±0.30	4.07 ±0.27	2.44 ±0.27	3.22 ±0.30	2.33 ±0.19
Basophiles (%)	0.60 ±0.13	0.70 ±0.08	0.78 ±0.08	0.88 ±0.12	1.03 ±0.14	0.66 ±0.10	0.58 ±0.14	0.97 ±0.17

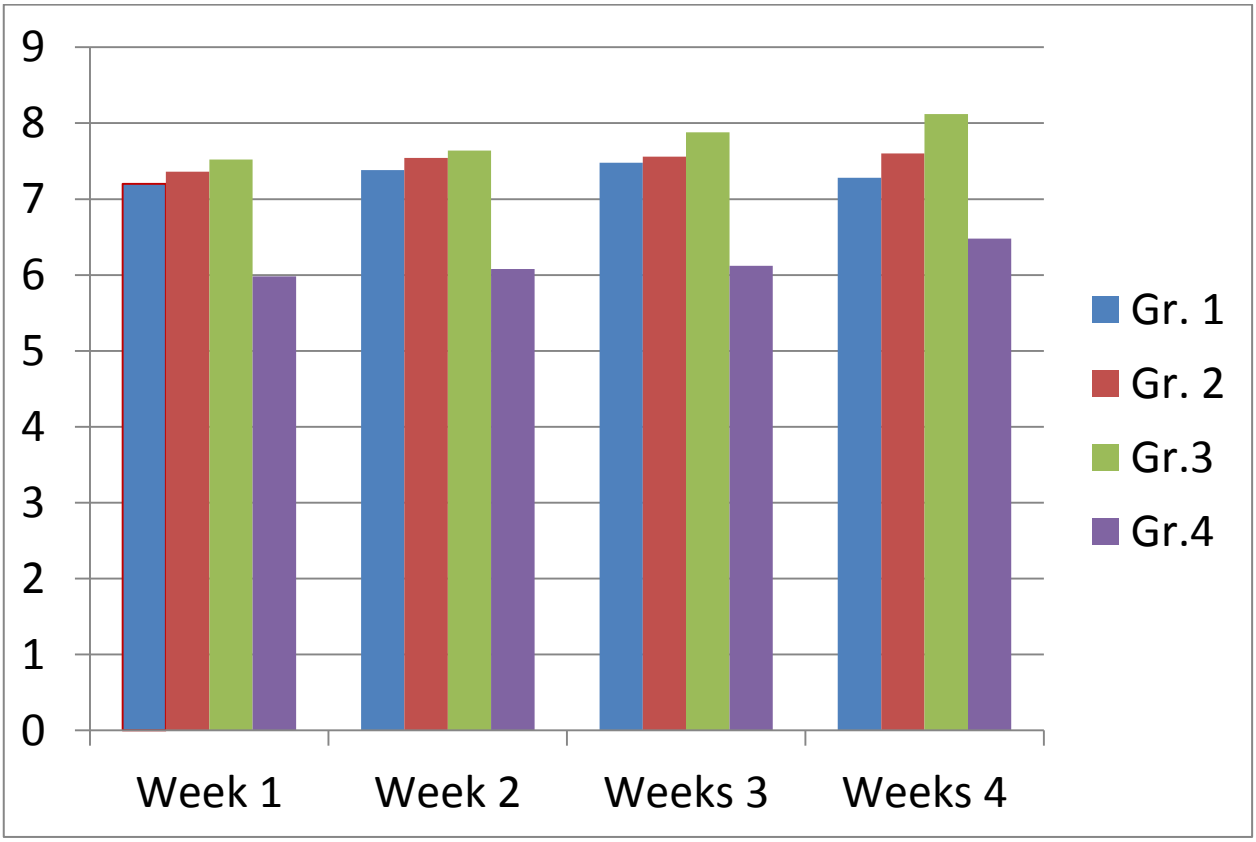
*****Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Table 4 :TLC (10^3 /mm^3) in control and CPF treated birds

Weeks post treatment	Gr. I	Gr. II	Gr. III	Gr. IV
1	18.16 ±1.38	19.98 ±1.37	20.88 ±1.38	17.74 ±1.28
2	24.26 ±1.29	25.22± 1.29	26.42± 1.76	22.30 ±1.14
3	30.36 ±1.19[a]	30.96 ±1.06[a]	32.60 ±1.07[a]	26.16 ±1.82[b]
4	33.14 ±1.70[a]	33.46 ±1.78[a]	34.20 ±1.63[a]	28.16 ±1.41[b]

*Means in a row having different superscripts differ significantly (P<0.05)

Fig. 2 : TLC of control and CPF treated birds



4.3. Biochemical studies

4.3.1. Alanine amino transferase (ALT)

From the Table 5, Fig No. 3 it is evident that CPF toxicity increase in ALT levels. The difference between the different treatment group was non- significant at 1st week At 2nd and 3rd week non- significant increase ($P<0.05$) in group I as compared to group IV which is control.

4.3.2 Aspartate amino transferase (AST)

The mean value of AST (IU/L) are presented in Table 6, Fig. No.4. The table shows that there is increased enzyme activity. The increase in activity of enzyme was significant ($P<0.05$) for different treatment level. The increase in level of enzyme was proportion to the treatment level. Between group II and III, increase was non – significant at 1^{st} , 2^{nd} and 3^{rd} week of exposure.

4.3.3 Alkaline phosphatase

The mean values of serum Alkaline phosphatase (IU/L) are presented in the Table 7, Fig. No. 5. Chlorpyrifos administration caused increase in serum alkaline phosphatise. The increase was non – significant at 1st and 2nd week of treatment. At 4th week, the increase was in proportion to the dose and time dependent. There was significant increase ($P<0.05$) in Alkaline phosphatase for all treatment group compared to control (Gr IV)

4.3.4 Total serum protein

The total protein values expressed as g/dl are represented in Table 8, Fig. No.6. The mean value of TP showed significant ($P<0.05$) increase in the treated group as compared to control.

4.3.5 Uric Acid :

Levels of mean uric acid (mg/dl) is presented in Table 9, Fig. No. 7. The increase in uric acid level was more pronounced at higher dose level. For the 1st two week of treatment, the increase was non-significant.

Ahmad *et al*., (2015) found increased activities of ALT and AST ($P\leq0.05$) in CPF treated birds which was time and dose dependent. Kammon *et al*., 2010 also found the activities of liver function enzymes viz. Alkaline phosphatase, ALT and AST were significantly increased in CPF induced chickens. Also Uric acid and glucose level was significantly increased.

The biochemical alteration in the serum indicated a wide range of degenerative or necrotic and inflammatory condition in parenchymatous organs, particularly in liver and kidneys in the CPF treated birds.

Table 5 :ALT(IU/L) of control and CPF treated birds

Weeks post treatment	Gr. I	Gr. II	Gr. III	Gr. IV
1	130.42 ±2.76^{c}	146.20 ±2.87^{b}	169.44 ±3.24^{a}	116.40 ±3.13^{d}
2	136.30 ±2.98^{c}	148.32± 2.30^{b}	164.24 ±3.98^{a}	11.36 ±2.46^{d}
3	138.20 ±2.33^{b}	146.30 ±2.70^{b}	160.16 ±3.49^{a}	125.52 ±2.83^{c}
4	134.60 ±2.13	148.62 ±2.31^{b}	162.20 ±2.38^{a}	115.30 ±2.65^{d}

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Fig. 3 : ALT (IU/L) of control and CPF treated birds

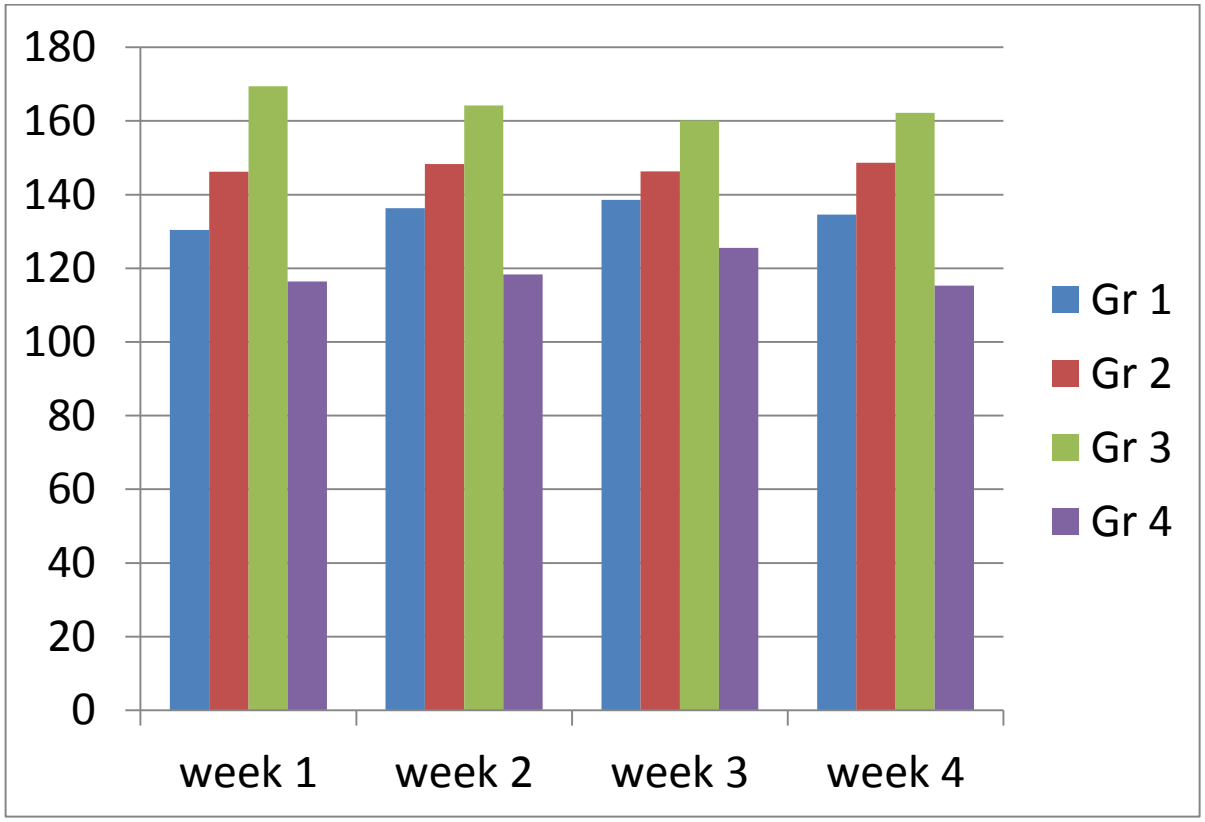


Table 6 : AST(IU/L) of control and CPF treated birds

Weeks post treatment	Gr. I	Gr. II	Gr. III	Gr. IV
1	6.75 ± 0.62	7.21 ±0.58	8.52±0.71	6.38 ±0.60
2	7.56 ±0.65^{b}	8.50±0.66^{b}	14.26 ± 0.61^{a}	6.10±0.57^{c}
3	8.20 ±0.77^{b}	17.22± 0.93^{a}	16.50 ± 1.16^{a}	6.80±0.55^{b}
4	12.46 ±1.34^{b}	16.24±1.27^{a}	19.28 ± 0.85^{a}	6.20 ± 0.53^{c}

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Fig. 4 : AST of control and CPF treated birds

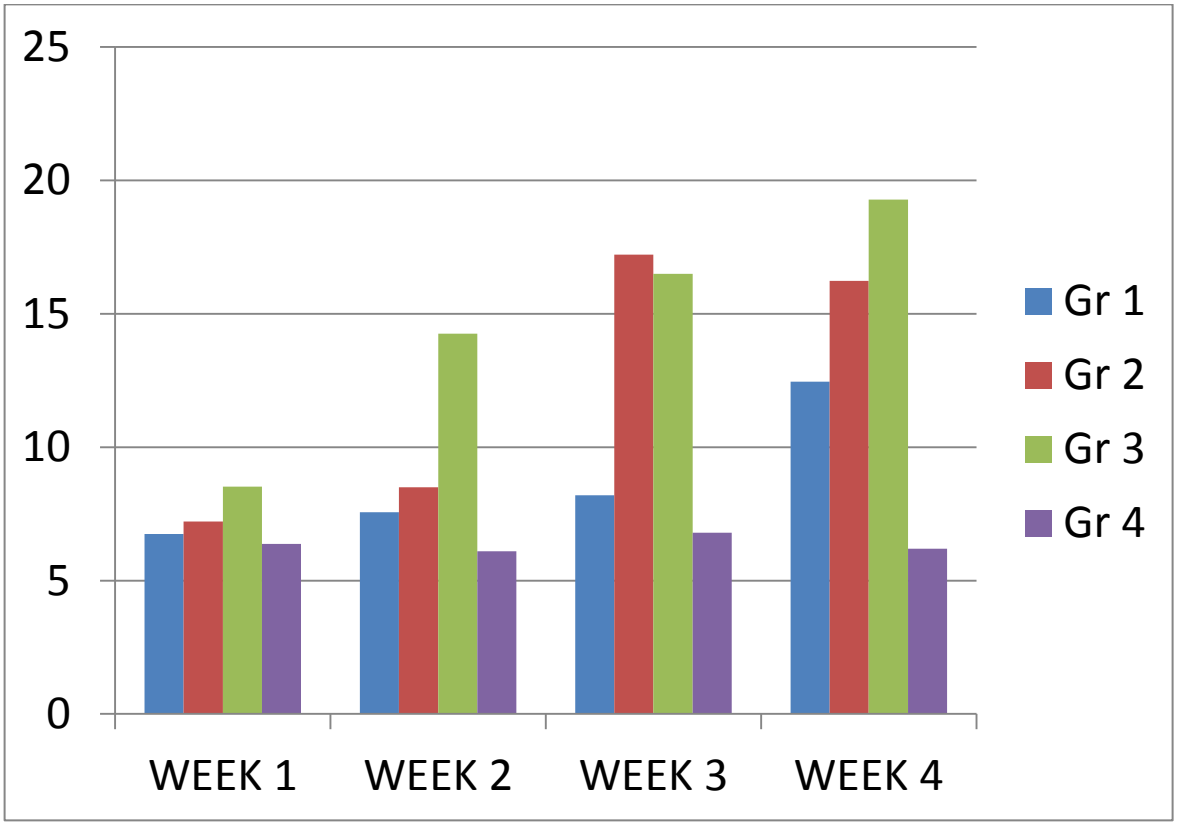


Table 7 : Alkaline Posphatase (iu/l) activity of different group of vanraja at weekly interval

Weeks post treatment	Gr. I	Gr. II	Gr. III	Gr. IV
1	20.30 ± 0.88	20.40 ± 0.95	21.80 ± 1.01	18.50 ± 0.86
2	21.54 ± 0.94	22.58 ± 1.11	23.60 ± 1.20	20.36 ± 0.85
3	23.72 ± 1.17^{c}	26.60 ± 1.10^{b}	32.10 ± 1.28^{a}	22.40 ± 1.02^{c}
4	25.44 ± 1.10^{b}	29.40 ± 1.32^{b}	34.32 ± 1.67^{a}	21.40 ± 1.04^{c}

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Fig. 5 : Alkaline Phosphatase of control and CPF treated birds

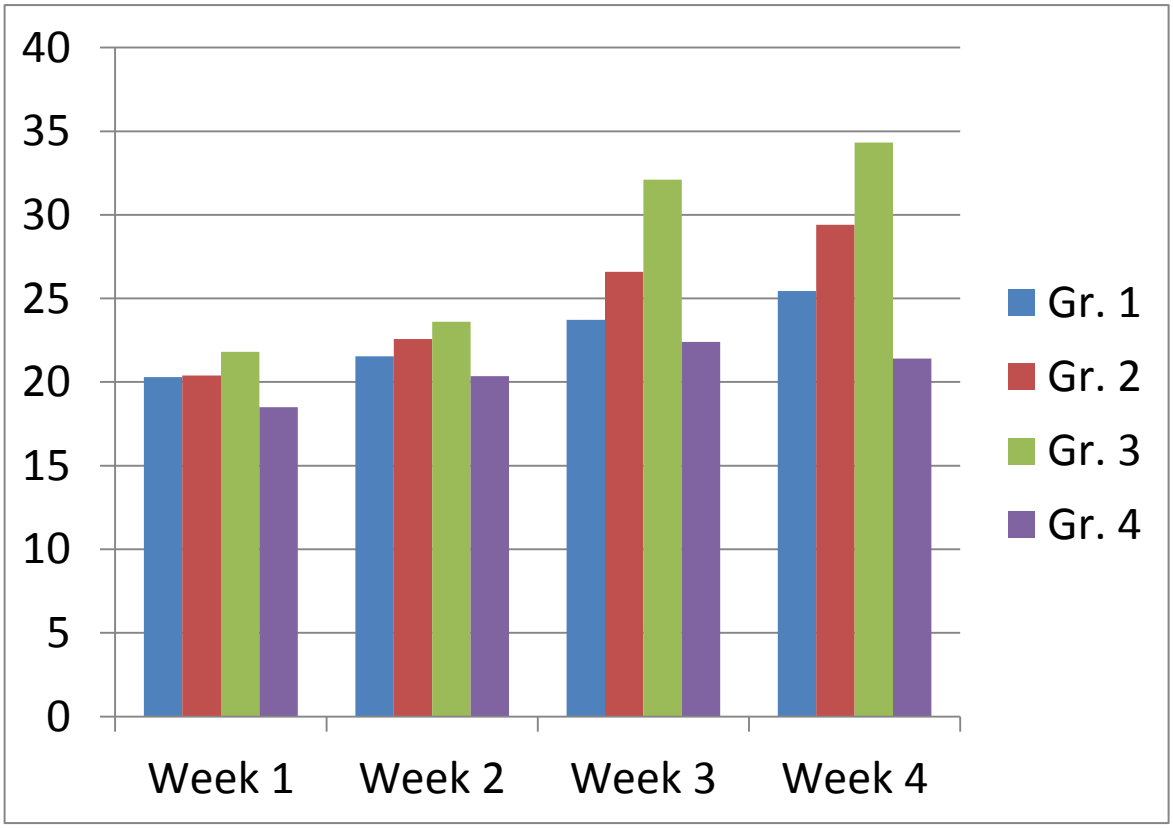


Table 8: Total protein (g/dl) of control and CPF treated birds

Weeks post treatment	Gr. I	Gr. II	Gr. III	Gr. IV
1	5.10 ±0.32[a]	5.12±0 .28[a]	5.03± 0.39[a]	3.60 ±0.24[b]
2	5.20± 0.33[a]	5.24± 0.29[a]	5.32± 0.37[a]	3.72±0.26[b]
3	5.30 ±0.36[a]	5.36± 0.34[a]	5.40 ±0.40[a]	3.91± 0.39[b]
4	5.18 ±0.30[a]	5.21 ±0.36[a]	5.74± 0.46[a]	3.68± 0.30[b]

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Fig. 6 : Total Protein of CPF Treated and Control Birds

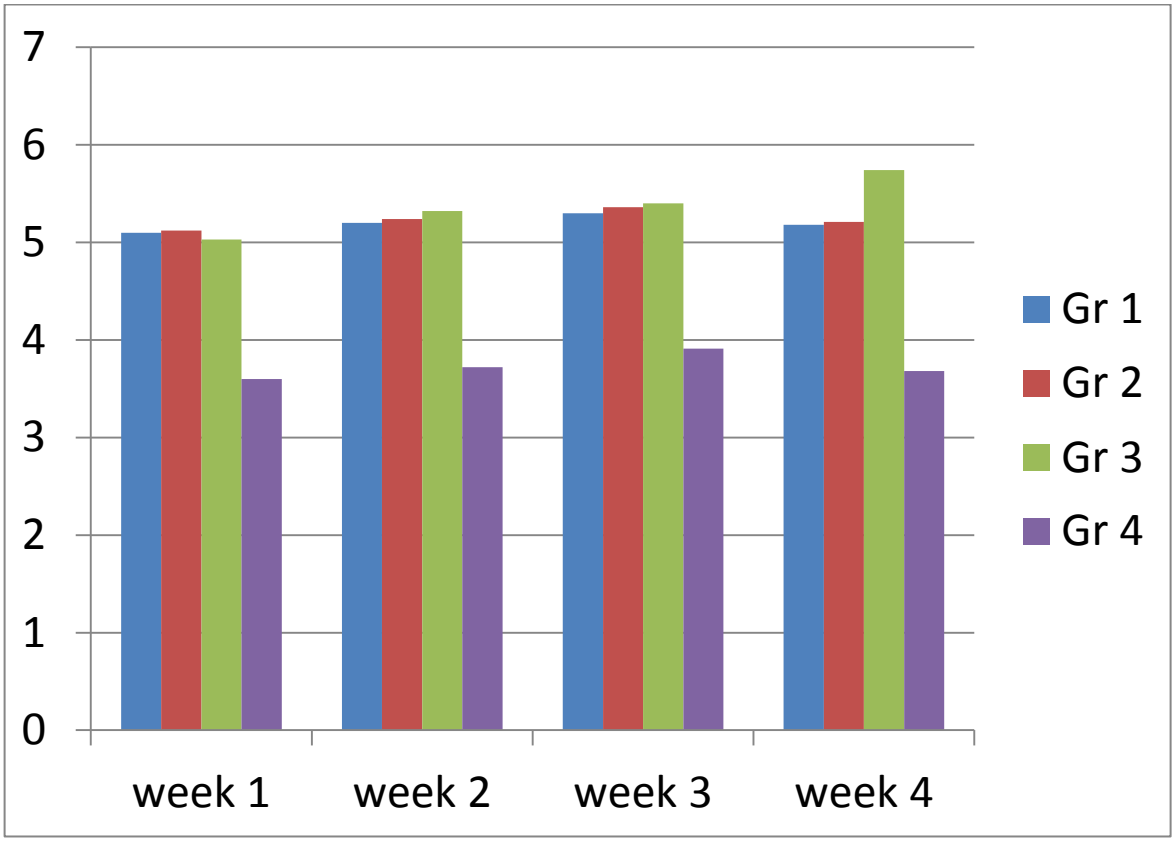
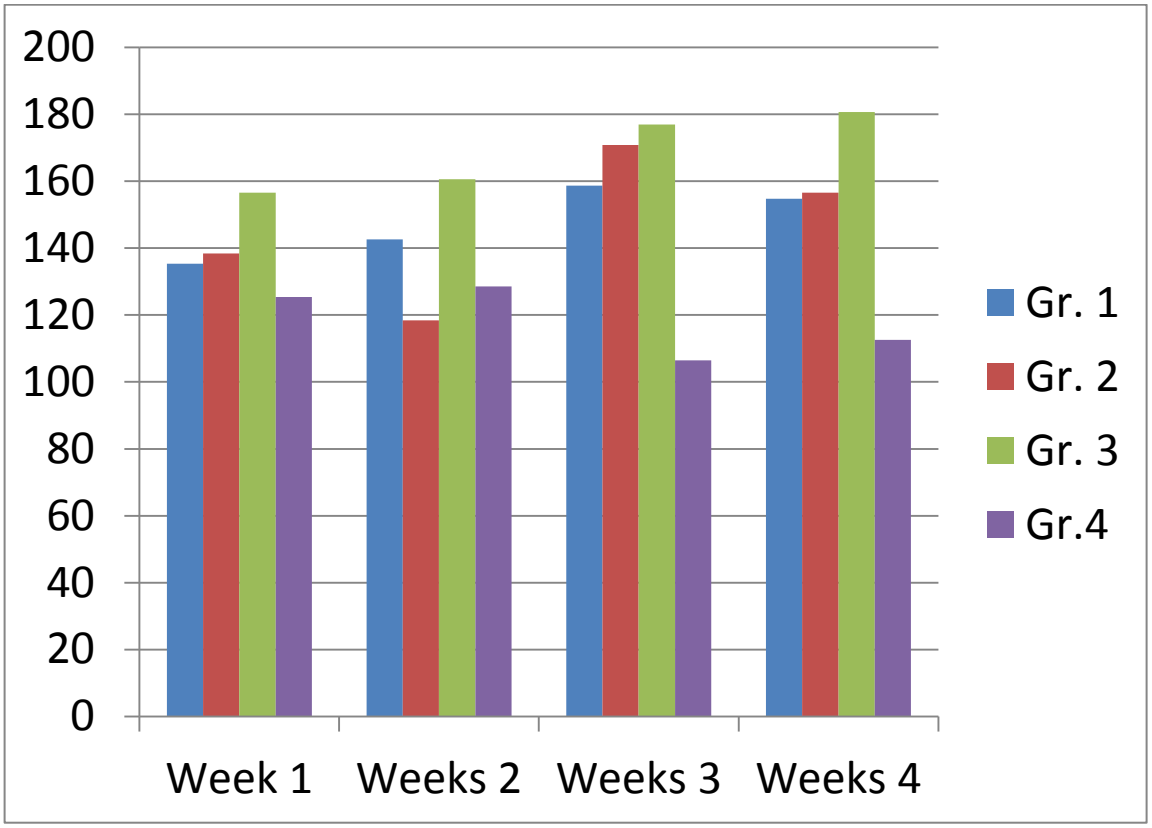


Table 9: Uric Acid(mg/dl) of different group of vanraja at weekly interval

Weeks post treatment	Gr. I	Gr. II	Gr. III	Gr. IV
1	135.36±4.92	138.4±4.74	156.60±4.70	125.38 ± 5.0
2	142.64±5.39	118.62±5.24	160.62±5.86	128.56±5.80
3	158.66±5.72^{b}	170.80±6.46^{a}	176.92±6.12^{a}	106.48±3.52^{c}
4	154.76±4.97^{b}	157.58±5.66^{b}	180.70±6.28^{a}	112.54±4.09^{c}

*Means in a row having different superscripts differ significantly (P<0.05; P<0.01)

Fig. 7 : Uric acid (mg/dl) in CPF treated birds and control



4.4 Pathomorphology

4.4.1 Grosspathology

Grossly, Gr II, III birds showed pale discoloration and enlargement of liver with severe haemorrhages. In Gr III birds, liver showed mild congestion, lungs were slight congestion and hemorrhages, intestines with petechial haemorrhages in serous layer week 4th week post treatment while haemorrhages on mucus layer in Gr II and III birds after 3rdweek post treatment. Both the kidneys were slightly congested and swollen in Gr III birds. Heart was slightly enlarged in Gr II and III birds and petechial haemorrhage on surface. Birds of Gr I showed no any visible lesions grossly in brain.

These results are in accordance with the results of Malik *et al*. (2002), Yadav *et al*. (2003), Sodhi *et al*. (2008), Kammon *et al*. (2010) and Yadav (2015), who observed similar changes in the parenchymatous organs of chicken induced with CPF toxicity.

Histopathology

Liver

Histopathologically marked degenerative changes occurred which were time and dose dependent. Lesion consisted of moderate to severe congestion in central and portal veins, hyperemia, cellular swelling with granular cytoplasm (Fig 8.), congestion in blood vessels, necrosis in the parenchyma and connective tissue proliferation between the lobule (fig 9)

sinusoidal dialatation, degeneration and coagulative necrosis of hepatocytes in the centrilobular and peripheral areas of liver i. e fatty change, fibrous tissue proliferation in portal triad (Fig 10.) in advanced stages of Gr III. birds. There was disorganisation of hepatic cords in Gr II and Gr III birds after 3rd week post treatment. Some of the liver section showed mononuclear cell infiltration. Similar lesions in the liver were reported by Malik *et al*., (2002), Tripathi and Srivastava, (2010) in CPF treated birds and by Sodhi *et al*. (2008) in CPF treated broiler chicks. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells.

Kidney

Microscopically, histopathological changes were evident in all treated groups from 2nd week onward which were times and dose dependent. It was congestion and extensive haemorrhages in the kidney tubules and infiltration of mononuclear cells in kidney (Fig.11), and condensation of nuclei of the tubular epithelium, degeneration and necrosis of the tubular epithelium (Fig.12) in advanced stage of toxicity. Mononuclear cell aggregation bcausing nodular structure, hypertrophy and hyperplasia of capillary ndothelium of glomeruli in high dose group. Desqumation of tubular epithelium and albuminous precipitation in lumen of tubules (fig.13) in all groups from 4th week post treatment. The histopathological findings of kidney in birds of all the groups of the present study corroborated with the findings of Kammon *et al*. (2010) in layer chickens and in broiler birds- Malik *et al*. (2002), Krishnamoorthy *et al*. (2007) and Kumar, (2011).

Heart

Histopathologically, there was mild histological alterations in myoca-rdium with loss of cross striation, fragmentation of myofibres and diffuse infiltration of mononuckear cells between muscle fibres (fig 14). The heart of the CPF treated birds revealed congestion and haemorrhage in blood vessels and separation of cardiac muscle fibres (fig 15). The histopathological changes found in the heart of CPF intoxicated birds in the present study corroborated the reports of the Kumar, (2011) in broiler birds.

Brain

Microscopically in the cerebrum, there was congestion of blood vessels (fig 16). Perivascular oedema and dialatation of Virchow-Robin space in all treated groups, more severe in Gr III. It was also focal and diffuse gliosis and necrosis of some neurons in different areas were evident. In the cerebellum, purkinje cells appear degenerated, infiltration of inflammatory cells (fig 17). Deplation of Purkinje cells were also noticed at some places. Mild oedema in Purkinje cell layer in advanced cases.

These reports are in agreement with findings of Malik *et al*. (2002) and Yadav *et al*. (2003) who reported perivascular and perineuronal oedema, gliosis and degeneration of a few neurons and Purkinje cells in broilers. Also the present study is in agreement with Krishnamoorthy *et al*. (2007) who found brain of CPF fed birds alone showed mononuclear cell infltration in meninges.

Lungs

There was congestion in birds of all treated groups more evidently in GrII and GrIII after 4th week post treatment. Some of atelectic alveoli with some emphysemated also, accumulation of serous exudates in alveoli with mono-nuclear inflammatory cells in perivascular areas of Gr. II birds. In birds of Gr. III proliferation of connective tissue between the alveoli and interlobular space in lungs (Fig 18).

The changes found in the lungs of chlorpyrifos treated birds in the present study is in agreement with the reports of the Kumar, (2011) in broiler birds.

Intestine

Intestine of Gr. II of birds showing atrophy of different layer of intestine (Fig 19) and birds of Gr. III showed mild to moderate haemorrhages and congestion and atrophy of different layer of intestine of Vanraja birds (Fig 20) after 4th week post treatment. There was necrosis of villi and goblet cell hyperplasia more intense in GrIII birds. Mononuclear infiltration in *lamina propria* .

The changes found in the intestine of CPF treated birds in the present study simulated the reports of the Kumar, (2011) in birds induced with chlorpyrifos.

Thus, from the present study it could be concluded that CPF toxicity is induced in birds also exhibiting nervous symptoms can be fatal also.

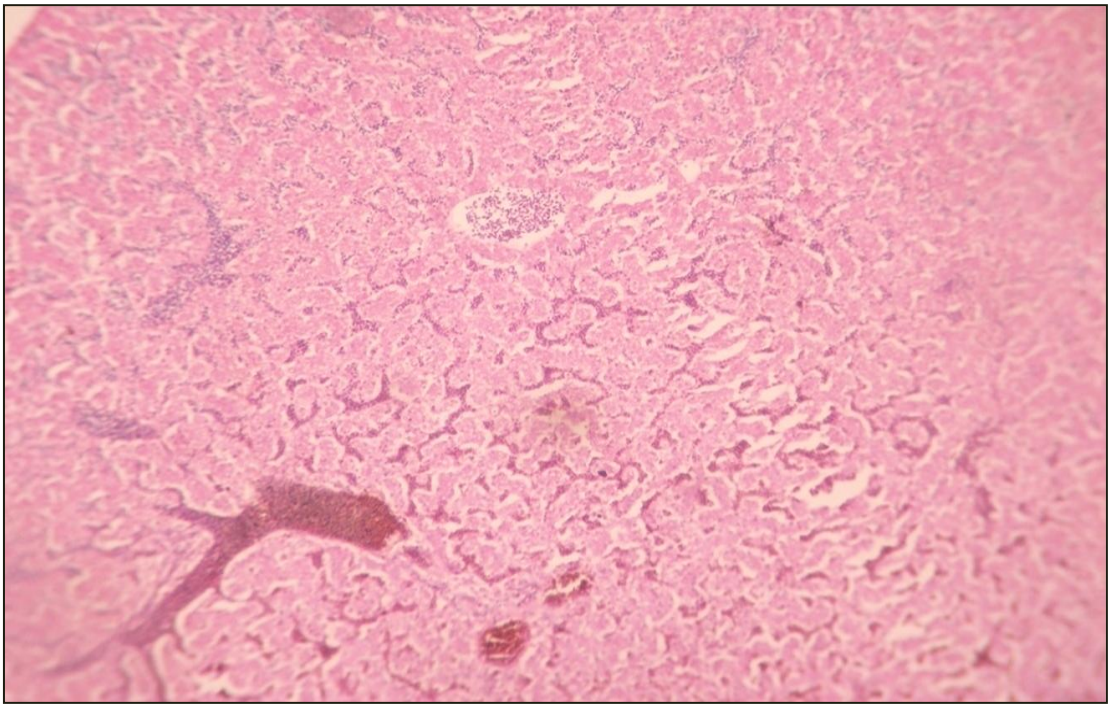


Fig. 8 : Microphotograph showing congestion in central and portal vein in Liver of vanaraja birds treated with chlorpyrifos 35mg/kg feed (H. & E.; X 40).

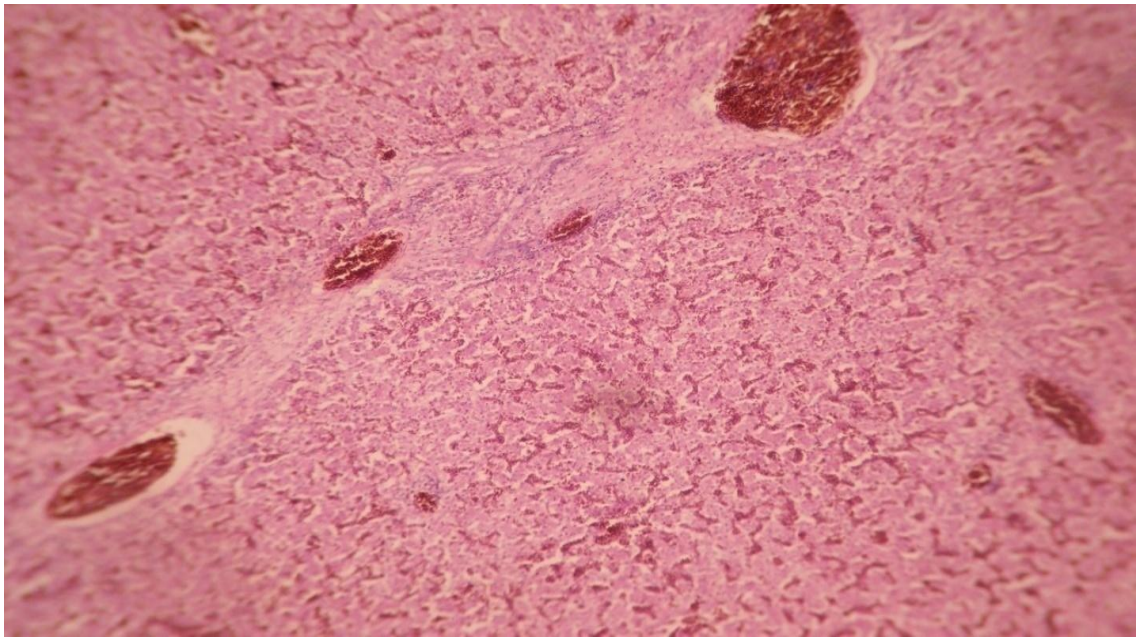


Fig. 9 : Microphotograph showing severe congestion in central and prtal vein evidenced by connective tissue proliferation between the hepatic lobules of Vanaraja birds treated with chlorpyrifos 70mg/kg feed (H. & E.; X 40).

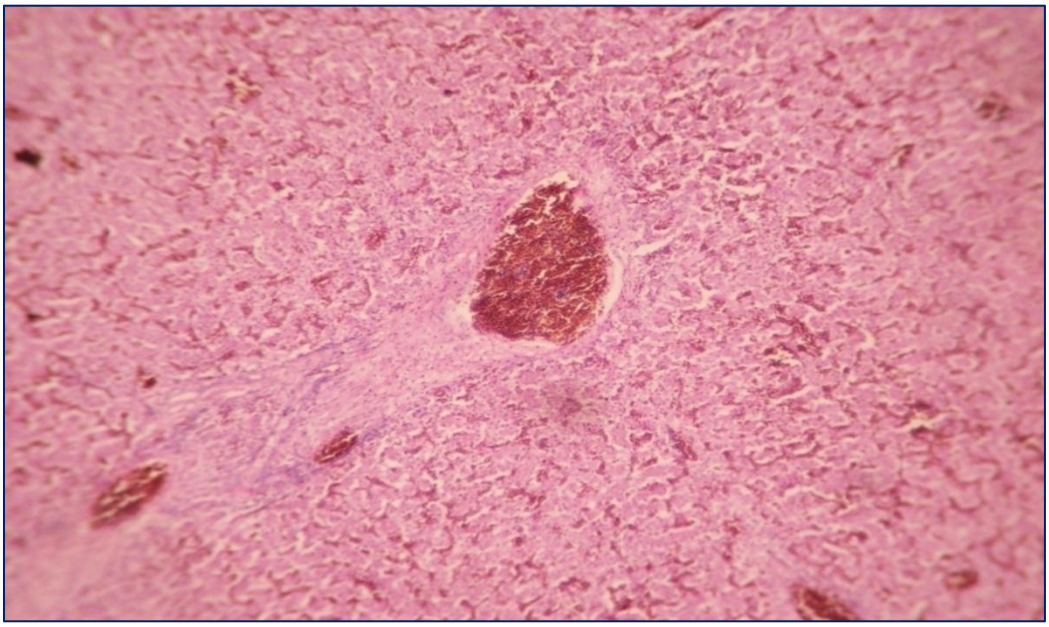


Fig 10: Microphotograph showing congestion in blood vessels, congestion in sinusoids, necrosis in the parenchyma and connective tissue proliferation between the lobule in Liver of Vanaraja birds treated with Chlorpyrifos 70mg/kg of feed (H. & E.; X 40).

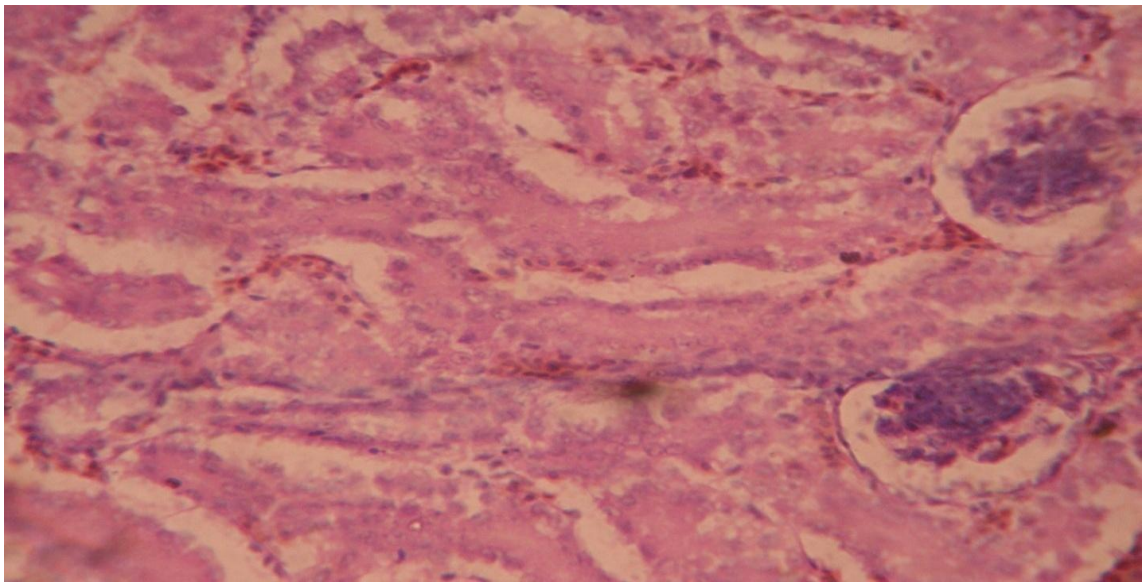


Fig. 11: Microphotograph showing tubular degeneration and infiltrations of mononuclear cells and congestion in Kidney of Vanaraja birds treated with chlorpyrifos 70 mg/kg feed (H .& E. ; x 450).

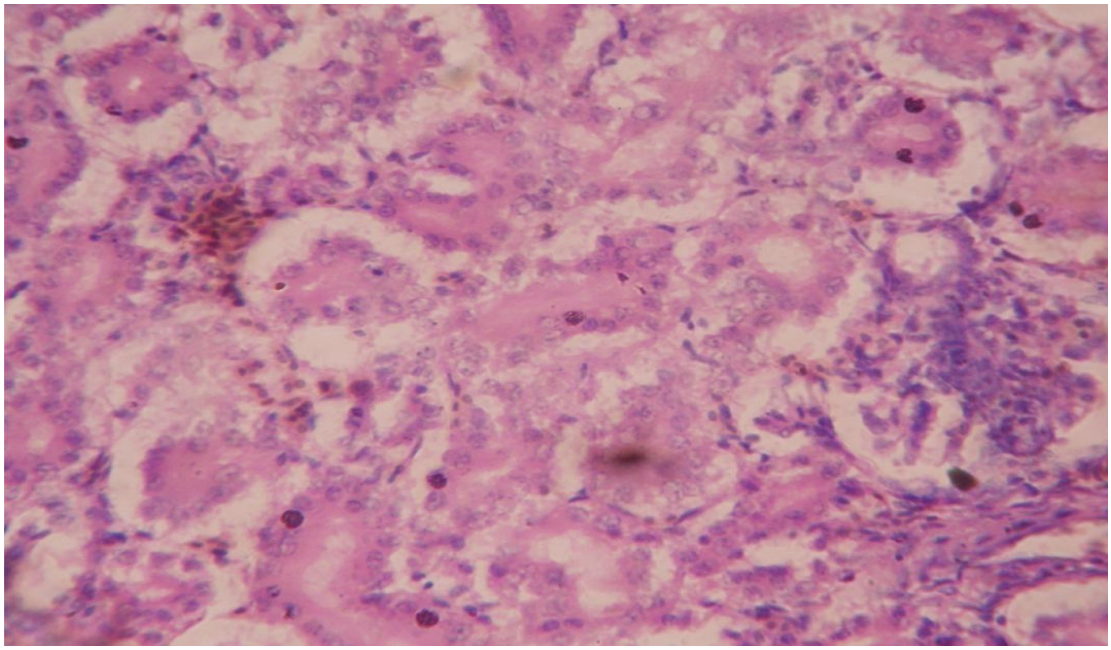


Fig. 12 : Microphotograph showing degeneration of tubules , Congestion and infiltrations of mononuclear cells of kidney of Vanraja birds treated with chorpyrifos 70 mg/kg feed(H .& E. ; x 100.)

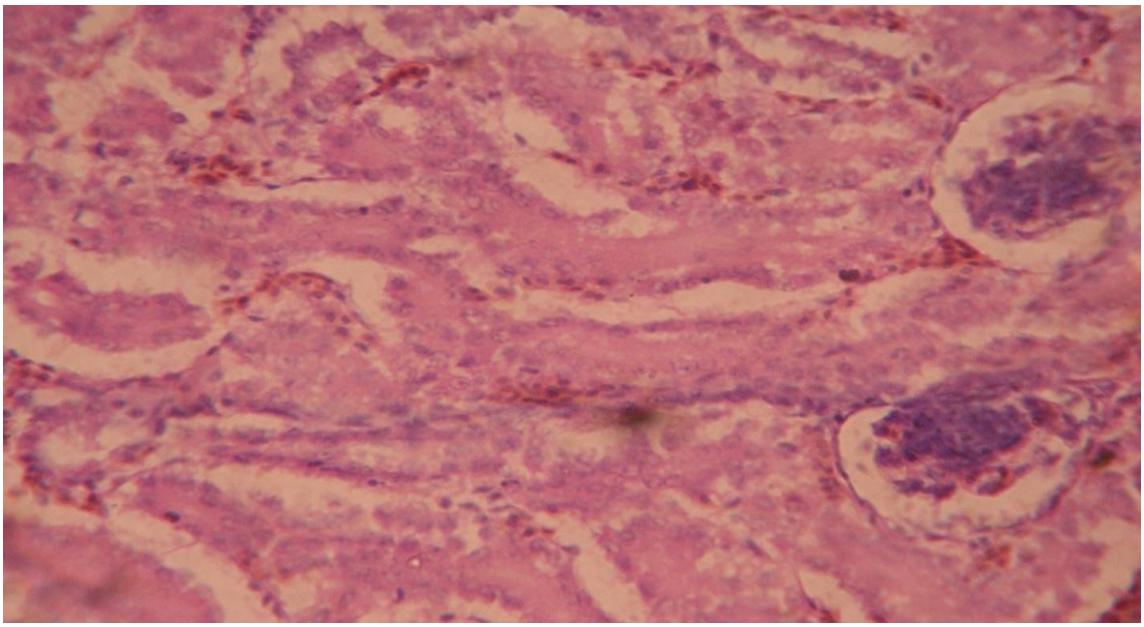


Fig. 13: Microphotograph showing tubular degeneration and infiltrations of mononuclear cells and congestion in Kidney of Vanaraja birds treated with chlorpyrifos 70 mg/kg feed (H .& E. ; x 450).

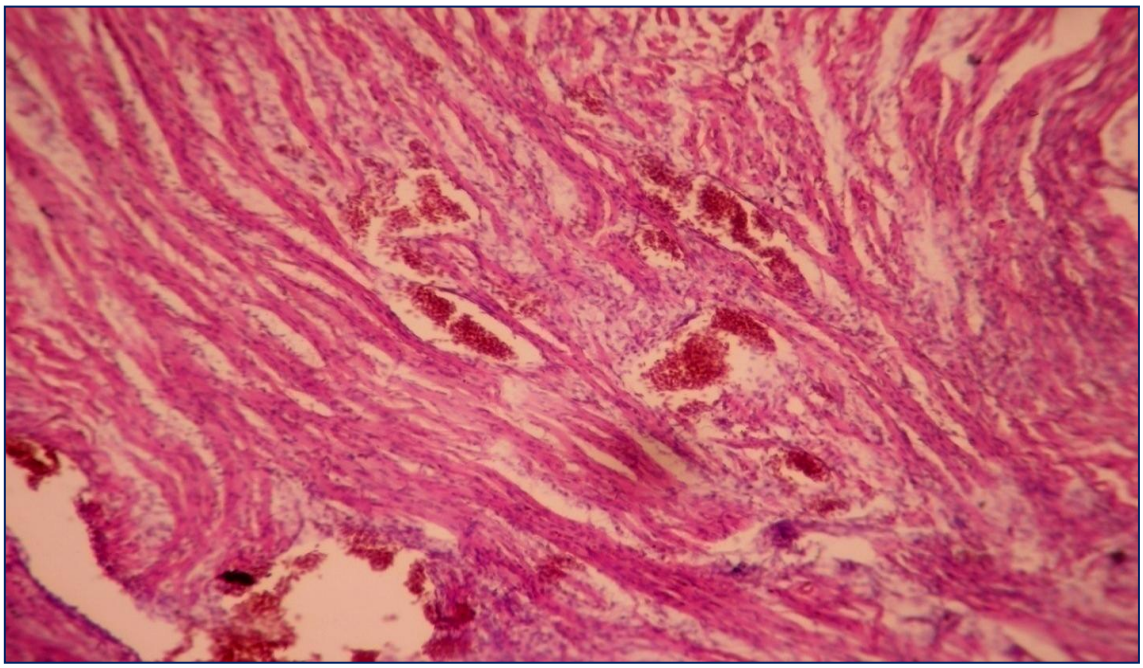


Fig. 14 : Microphotograph showing degenerative changes and infiltration of mononuclear cells in cardiac muscle of vanaraja birds treated with chlorpyrifos 70mg/kg feed (H. & E.; X 100).

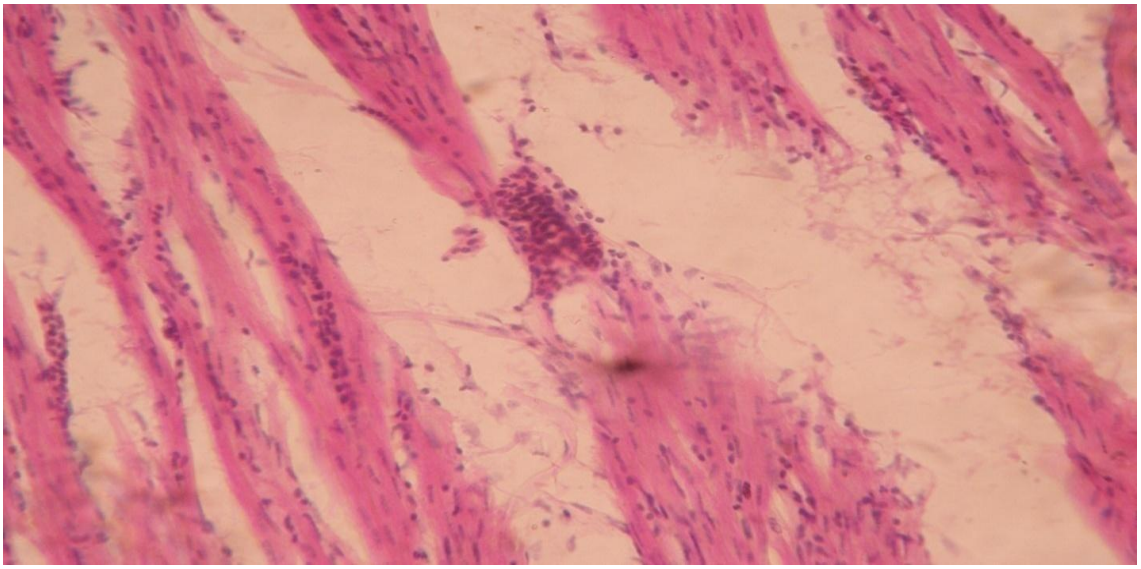


Fig. 15: Microphotograph showing Conjestion & Haemorrhage in blood vessels & cardiac muscles of Vanaraja birds treated with chlorpyrifos 140 mg/kg feed (H .& E. ; x 40).

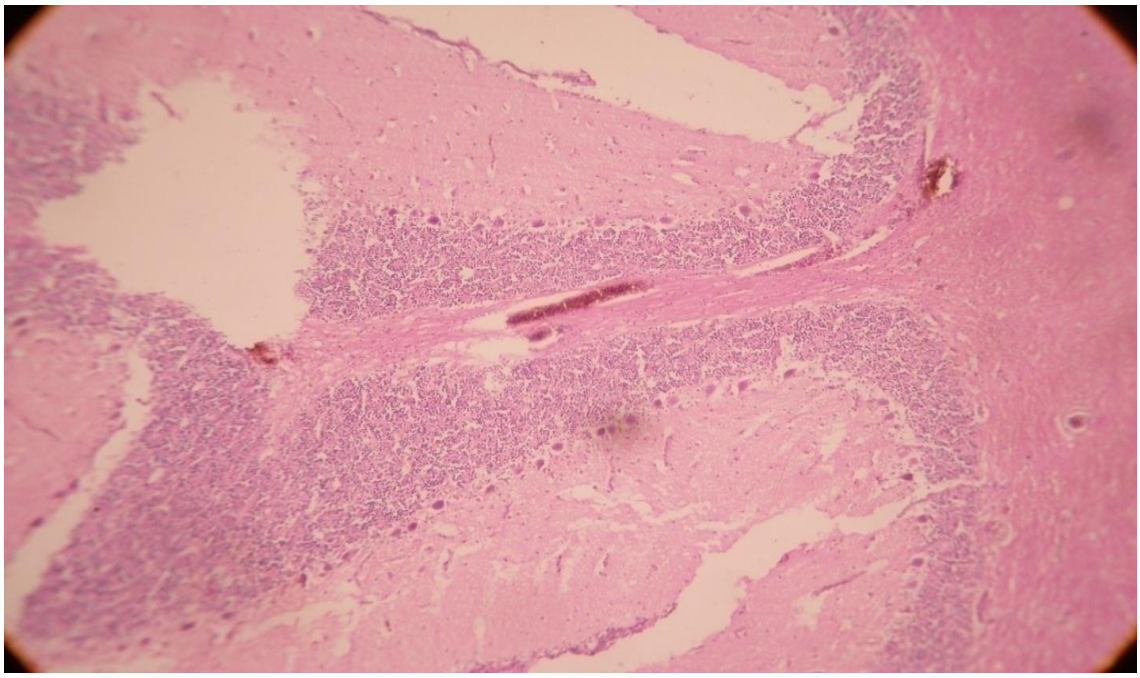


Fig. 16: Microphotograph showing congestion of blood vessels of the brain of Vanraja birds treated with chlorpyrifos 70 mg/kg feed (H .& E. ; x 100.)

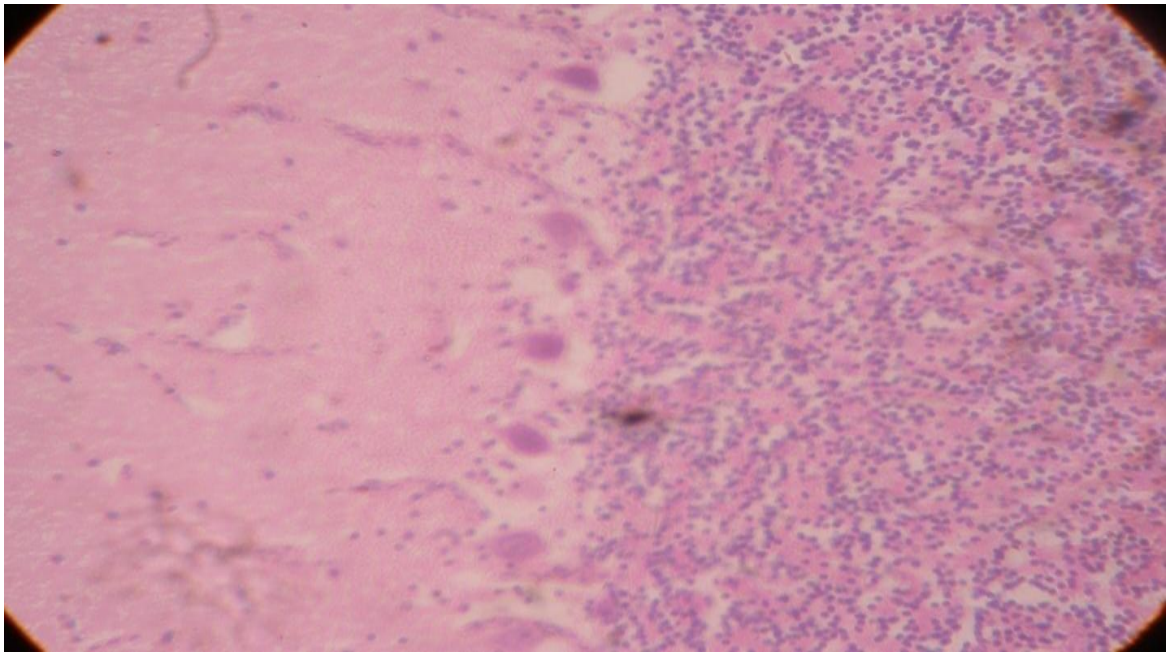


Fig. 17: Microphotograph showing degeneration of purkinje cells and inflitration of inflamatory cells of brain Vanaraja birds treated with chlorpyrifos 70 mg/kg feed (H .& E. ; x 450.)

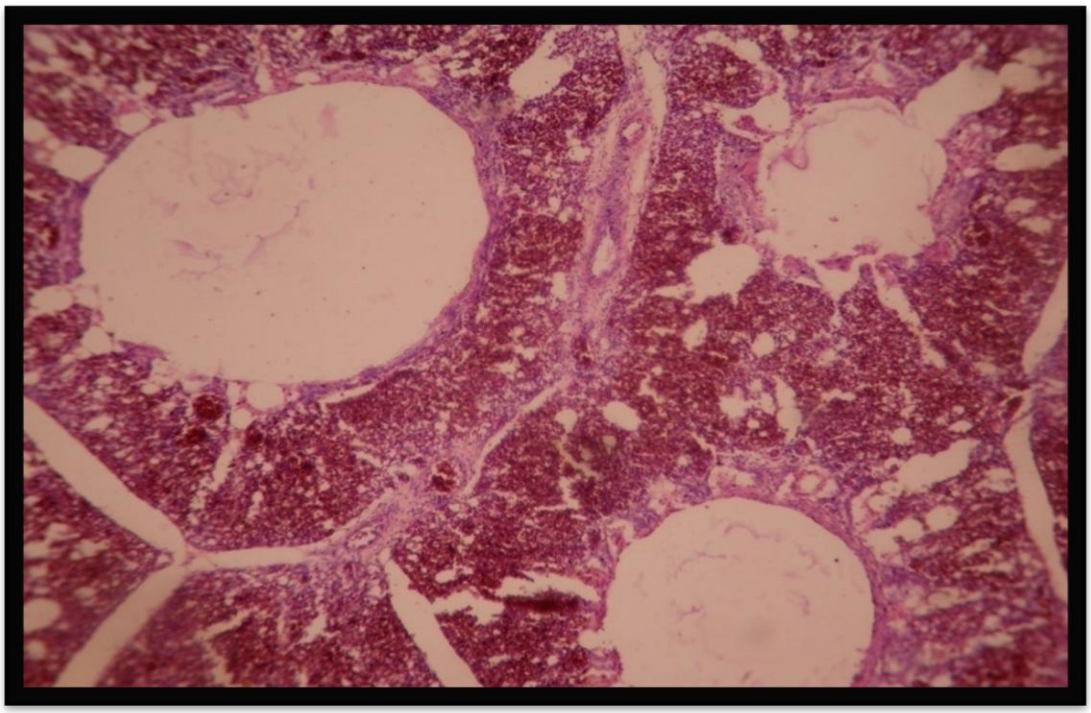


Fig.18: Microphotograph showings proliferation of connective tissue between the alveoli and interlobular spaces in Lung of Vanraja birds treated with Chlorpyrifos 140 mg/kg feed H. & E. ; X 450.

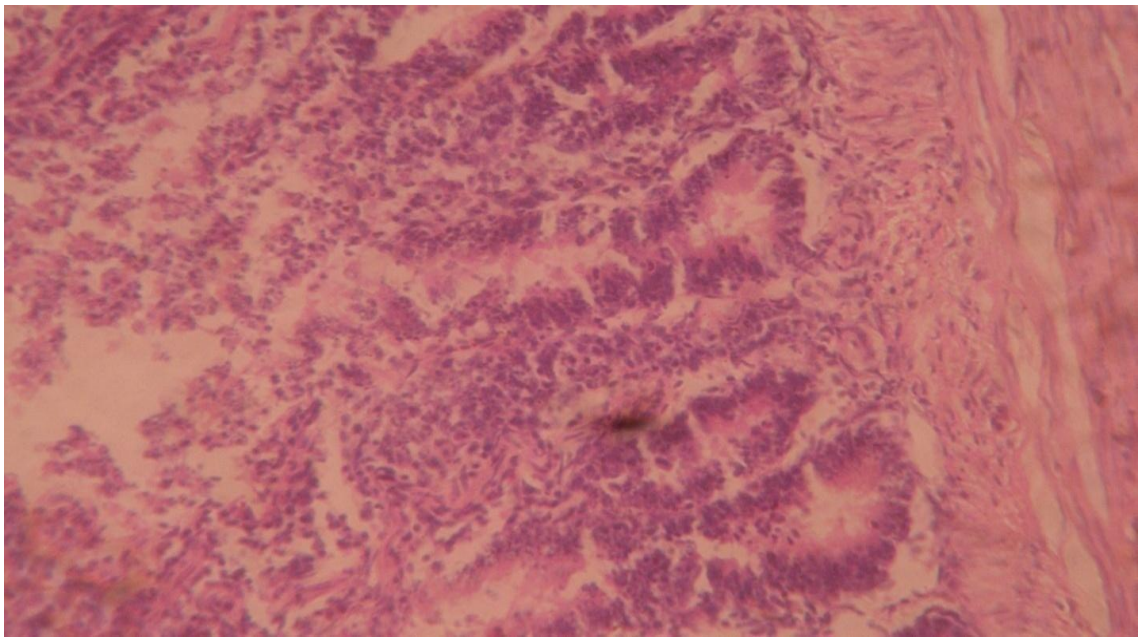


Fig. 19: Microphotograph showing atrophy of different layer of intestine of Vanraja birds treated with chlorpyrifos 35mg/kg feed. (H. & E. ; x 450.)

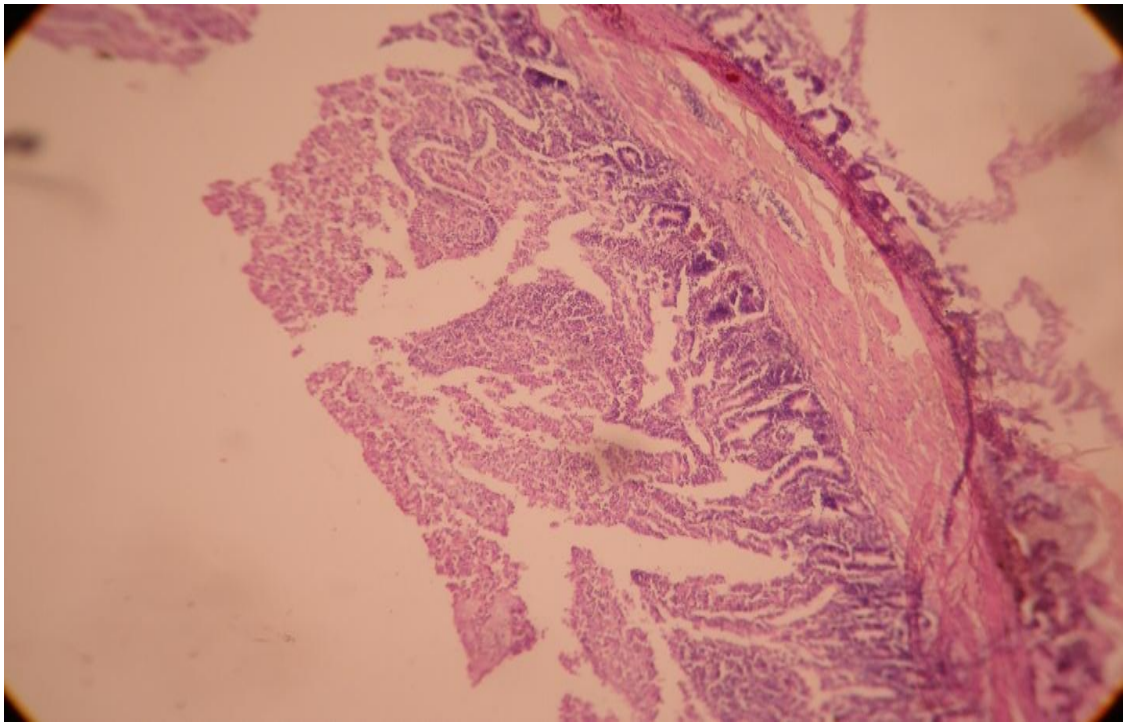


Fig. 20 : Microphotograph showing atrophy of different layer of intestine of Vanraja birds treated with chlorpyrifos 140 mg/kg feed (H. & E. ; x 100.)

5 SUMMARY AND CONCLUSIONS

Organophosphate insecticides are one of the most widely used chemicals in agriculture and public health. Chlorpyrifos, an organophosphate (OP) insecticide, induces neurotoxicity and tissue damage with observable signs of poisoning. The primary mechanism of toxicity is associated with its ability and especially that of its metabolite, CPF-oxon to inhibit acetylcholinesterase (AChE), an enzyme that normally terminates neuro- transmission at cholinergic synapses. Prolonged exposure to chlorpyrifos has been shown to cause severe damage to the vital organs. In the present study, we investigated the chlorpyrifos-evoked haematological, biochemical and related histopathological alteration in Vanraja birds upon 2weeks and 8 weeks exposure have been correlated with the different dilutions of LD50 of chlorpyrifos administered.

The birds show decrease in body weight during trial but it was insignificant. Body weights of CPF induced chickens GrI, II, III were significantly ($P<0.05$) lowered from 3 weeks onwards when compared with the control chickens .

CPF poisoning caused severe diarrhoea causing fluid loss, inappetance and a gradual loss of general condition. Reduced appetite, frequent diarrhoea along with the damage of liver, kidney and gastro-intestinal tract due to effect of CPF might have played an important role in body weight loss .

Results of hematological findings are ($P<0.05$ or $P<0.01$) increase in Hb, PCV and TEC in CPF induced Vanraja birds compared to control birds after 2 and 4 weeks of treatment. Gr III birds showed significant improvement when compared to that of Gr II but the values were higher than that of the Gr IV. The rise in Hb concentration, PCV and TEC in the present study might be due to the dehydration resulting in diarrhoea and excessive salivation. A relative decrease in lymphocyte count and increase in neutrophil count was significant ($P<0.05$). The lymphopenia observed in the CPF intoxication may be due to either the decreased production and/or increased rate of removal due to rapid destruction. The activities of liver function enzymes viz. Alkaline phosphatase, ALT and AST were significantly increased in CPF induced chickens. Also Uric acid and glucose level was significantly increased.

The biochemical alteration in the serum may be due to wide range of degenerative or necrotic and inflammatory condition in parenchymatous organs, particularly in liver and kidneys in the CPF treated birds.

Histopathological changes in visceral organs. Liver lesion consiste of moderate to severe congestion in central and portal veins, hyperemia, cellular swelling with granular cytoplasm. In Kidneys, there was

congestion and extensive haemorrhages and infiltration of mononuclear cells. The heart of the CPF treated birds revealed that congestion and haemorrhage in blood vessels and separation of cardiac muscle fibres. Brain perivascular oedema and dialatation of Virchow-Robin space in all treated groups, more severe in Gr III. Congestion in lungs of birds of all treated groups more evidently in GrII and GrIII after 4^{th} week post treatment. Intestine of Gr. II of birds showing atrophy of different layer of intestine and birds of Gr. III showed mild to moderate haemorrhages and congestion and atrophy of different layer of intestine of Vanraja birds.

6. FUTURE SCOPE OF RESEARCH

It is virtually impossible to cover all toxico-biological effects of chlorpyrifos due to limitation as regards of facilities and due to obvious reason of time boundness. Therefore, the following parameter should be considered in the future plan of research programme.

(i) The response of humoral and cell mediated immunity in birds induced with CPF at different dose levels should be assessed

(ii) The detailed histochemical features of Vanraja birds induced with CPF should be studied.

(iii) The residual concentration of CPF in organ/tissue should be studied.

(iv) Feed conversion ratio and relative organ weight on weekly basis should be assessed.

(v) More intensive studies are suggested to evaluate the safety of the CPF and its pathophysiological implication in the avian system.

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