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ANALYSIS OF TOXIC IMPACTS OF FIPRONIL ON GROWTH, HAEMATOLOGY ANDOXIDATIVE STRESS IN CHICKEN

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Abstract
Fipronil (FIP), which is used globally to control pests and also as veterinary drug is a broad-spectrum phenylpyrazole. The excessive and uncontrolled use of FIP causes poisoning of soil and water that could have toxic impacts on targeted and non-targeted organisms as well. So regarding to this, our current study was designed, mainly to examine the toxic impacts of different concentrations of FIP in chicken. Fipronil preparation was prepared by mixing it with corn oil and given orally to different groups as follows: A (control), B (0.25 mg/ml/kg), C (0.5 mg/ml/kg), and D (0.75 mg/ml/kg)for 30 days. Signs of toxicity like anemic comb, tremors, gasping and watery droppings were observed in birds, exposed to higher levels. The feed intake, body weight, absolute and the relative weight of different organs were significantly decreased in birds, exposed to higher concentrations. Growth analysis of birds exposed to higher levels of FIP showed decreased weight as compared to the control group. The Oxidative stress analysis resulted in an increase of LPO in both kidneys and liver while catalase was decreased in kidneys and liver. Moreover, significant (p_.05) decrease in hematological parameters such as Red blood cells counts an hemoglobin concentrations, while increase in total leukocyte counts and platelets were recorded in birds, however other hematological parameters as HCT,MCH,MCHC and MCV remained almost same. Furthermore, significant increase in concentrations of serum urea, creatinine, alanine amino transferase,an aspartate amino transferase were also recorded in birds. The inferences we have made from our study clearly indicated that Fipronil has lethal and toxic impacts on various tissues of birds from high to even low doses.
Key words: Fipronil, Growth, Haematology, Oxidative stress, Chicken

INTRODUCTION

Poultry sector is an important segment in the provision of protein (meat and egg) by rearing domesticated species. It has a great importance in the national economy i.e. having a remarkable contribution in the Pakistan GDP as of 1.3%. It is started in 1960s in Pakistan and is serving as a daily

protein source for the Pakistani population (Hussain *et al*., 2015).Chicken eggs are the primary source of vital nutrients, especially for low-income individuals living in developing nations (Farrel, 2009). The ruminant or other larger animals have meat with high fat and cholesterol level as compared to poultry meat (Al-Khalifa *et al*., 2014; Zdanowska-S ˛asiadek*et al*., 2018). The poultry meat is known to be the cheapest source of animal proteins for human beings regarding to its cost and availability (Drewnowski *et al*., 2010; Lesnierowski, 2018).

Diverse waste products from various agricultural activities are continuously released into the enviro nment and have a negative impact on living creatures (Husain et al., 2014; Chaguri et al., 2016; Jan et al., 2017). With the development of industry and technology in every field of life such as agriculture or others, we also became habitual to use synthetic chemicals such as pesticide, herbicide and insecticide which are causing lethal impacts on soil and all parts of ecosystem (Hussain *et al*., 2015; Ghaffar *et al*., 2021).

Poultry manure has a definite amount of liquid that gives a perfect habitat for large population of house flies (Szalanski *et al*., 2004). House flies are a source to transmit Campylobacter to the broiler houses. They pick up the Campylobacter by visiting places where cattle and poultry are kept, then they bring it to the broiler house. As these flies population reach at its peak in summer. Infections with Campylobacter are more common throughout the summer (Patrick *et al*., 2004; Hanson *et al*., 2007). It is a cause of the transmission of poultry diseases. It was seen as a source for the virus's transmission, which started the epidemic of Newcastle disease in the 1970s (Watson *et al*., 2007). House flies are more sensitive to fipronil derivatives (Hainzl*et al*., 1998). The experiment shows a correlation between the potency of phenylpyrazoles and other GABA-gated chloride channel blockers and their toxicity to house flies (Deng*et al*., 1991). Fipronil (FIP) is a phenylpyrazole pesticide. Many types of insects includes ticks, termites, rootworms, weevils, cockroaches, ant, flies and beetles can be controlled by using fipronil (Tomlin, 2006). It was uncovered by Rhone-Poulenc Agro in 1987, commercialized in 1993, and registered in the US in 1996 (Aajoud*et al*., 2003). It can be used in solid form (e.g., insect bait), also as a liquid spray, or in granular form (e.g., turf application), but granular form of fipronil is most persistent. It favours lipophilic (organic) that is more stable at room temperature. It has a range of solubility in water from moderate to high (Aajoud*et al*., 2003), but only for one year and not stable in the presence of metallic ions. It is degraded by sunlight and change into its metabolites, fipronil-desulfinyl is one of these metabolites which is more stable and toxic than its parent substance (Das *et al*., 2006). Being a substance of phenylpyrazole, fipronil causes the blocking of GABA receptor, it causes oxidative stress by producing the receptive oxygen species (Vidau *et al*., 2011; Hussain *et al*., 2014; Khan *et al*., 2015). It has been discovered that fipronil reduces hatching success and causes mothers to pass on fipronil residues to their offspring when they lay eggs (Kitulagodage*et al*., 2011). It has an adverse effect on the visceral organs such as testes, liver and thyroid in the non-targeted species (Ohi *et al*., 2004; De Oliveira *et al*., 2012).

It is also an endocrine disruptor mediator that cause many harmful health effects such as behavioral, reproductive and developmental threats (Udo *et al*., 2014; Badgujar *et al*., 2015). Fipronil cause the exchange in chromatids, DNA damage and also the micronuclei damage in the human lymphocytes. It also causes the change in all blood parameters as Hb, WBCs, RBCs, HCT, MCV, MCH, MCHC an platelets (Celik *et al*., 2014). Fipronil cause a decrease in the body weight. A prominent decrease in the weight of internal body organs also observed in other species (Hussain *et al*., 2018). The present study designed to check out the toxic impacts of fipronil on growth, hematology and oxidative stress in chicken.

MATERIALS AND METHODS

Study animal

Total 80 samples of similar age (40 days) and of almost similar body weight, were collected.

Housing management

All Chicks were kept under strict sanitation system. They received immunizations to protect them from several viral infections including infectious bronchitis and infectious bursal disease. The typical

management conditions of temperature (26-280C) and humidity (62-67%) were provided to them. All chickens were raised in the same manner.
Before housing the chickens, experimental room was thoroughly cleaned to prevent them from the effects of germs.

Feed
The chicks were fed on basal control diet (i.e. commercial feed) and this feed was provided with clean tap water. Rice, corn and oat were also provided.

Experimental protocol
The birds were randomly separated into four groups with 20 birds in each group, after two weeks of acclimation. One group was taken as control group which was not exposed to the chemical and supposed to be the group of healthy chickens, while other three groups II, III and IV birds were given different concentration of fipronil. Different concentrations of fipronil was given orally in 100ml of water to all the groups such as A=control group without chemical dose, B=0.25mg/ml/kg body weight, C= 0.5mg/ml/kg and D=0.75mg/ml/kg. 2ml was given to each of the bird orally with help of syringe. All birds were clinically healthy at the start of the study without any sign of any disease.
All treatment groups were treated for 30 consecutive days with low, moderate and high concentrations of fipronil.

Chemical
The standard solution was made by dissolving its different concentrations in the distilled water i.e.0.25mg/ml/kg, 0.50mg/ml/kg and 0.75mg/ml/kg in 100ml of distilled water. Birds of all groups were exposed to 2ml of solution.

Assessment of growth
Growth was examined by measuring the body weight. This was done by using weight balance. Weight measured at first day as in the start of experiment, followed by the mid of experiment as after 15 days and at the end as after 30 days.

Hematology procedure
Birds were handled carefully to reduce the stress. Blood samples about 5ml were collected with the help of plastic syringe from brachial wing and then transferred to anticoagulant EDTA (1mg/ml) tube which prevent blood to coagulate. Different blood parameters such as Hb, HCT, RBCs, MCH, WBCs, MCV, MCHC and platelets were examined. By centrifuging blood at 2700rpm for 15 min at 4°C, serum samples were extracted and split into Eppendorf tubes. Isolated sera were kept at -20°C in storage. Different parameters such ALT, ASP, urea and creatinine were examined spectrophotometrically (Ghaffar *et al*., 2015)

Oxidative stress assessment
Birds were slaughtered carefully and different body organs such as liver and kidney were isolated and centrifuged at 1000rmp after that supernatants were separated and used for evaluation of antioxidant activity. By using the (Ohkawa *et al*., 1971) technique, lipid peroxidation (LPO) was observed. Catalase (CAT) activity was performed by the method of (Goth, 1991).

Statistical analysis
Data was analyzed statistically by one way analysis of variance (ANOVA) followed by post hoc Tukey's testto determine the toxic effect of fipronil on selected parameter of chicken samples. Resultwere expressed as mean±standard deviation.

RESULTS

Clinical signs

Behavioral changes were observed during the different stages of the experiment. Some behavioral changes were observed exactly at the time of insecticide exposure and some were observed during the period of experiment gradually. Some behavioral changes that were noticed within an hour of administration of different concentrations of fipronil included tremors, hypersalivation, nervousness and ruffled feathers. During the experimental period, some other clinical and behavioral signs such as watery feces, inappetence, dullness, decrease in feed intake and body weight, depression, weakness, decrease in crowing frequency, anemic comb, and gasping were also noticed.

Growth analysis

Owing to repeated doses of fipronil, a significant decline in feed intake was observed at the 15^{th} day of the experiment, specifically in those groups which were exposed to higher FIP concentration intake. The insecticide fipronil influenced on the growth of*Gallus gallus domesticus*. The (Table1) shows that there was a significant difference in the weight gain in control and treated group. After15 and 30 days of fipronil exposure, the rate of weight gain was decreased as compared to control group. A considerable loss in weight of chicks was observed in group D due to high concentration of fipronil, as compared to group B, C and control group A. It has also been shown in Fig 1. After 30 days exposure to fipronil birds were dissected, blood sample and different body organs were collected for the analysis of hematology and oxidative stress.

Analysis of serum biochemical parameters

The Concentrations of ALT, AST, urea and creatinine has been shownin (Table 2). The high level of ALT, AST, urea and creatinine was observed at high concentration of fipronil in group D followed by group B, C and control group A as shown in Fig 2. A statistically significant difference ($p<0.005$) in the value of enzyme ALT, AST, urea and creatinine was observed.

Hematological studies

Hematological findings after 30 days of experiment, exhibited a clear change in the level of hematological parameters. A significant increasein WBCs and platelets level and a decrease in the level of RBCs and hemoglobin, at high concentration in group D followed by group B, C and control group A has been shown. However some other hematological parameters such as HCT, MC, MCH, and MCHC observed at same level in all the groups as shown in Fig 3.

Oxidative stress analysis

LPO and CAT were observed in kidneys and liver, after exposure to fipronil for 30 days. A considerable rise in LPO and significant decrease in CAT level was observedin both kidneys and liver in group D followed by C, B and control group A as shown in (Table 4) and Fig.4.

DISCUSSION

Fipronil (FIP) is the N-phenyl pyrazole insecticide with a trifluoromethylsulfonyl moiety (Tingle *et al*., 2003; Mohamed *et al*., 2004; Wu *et al*., 2015). The International Union of Pure and Applied Chemistry (IUPAC) name for fipronil is (±)-5-amino-1-(2, 6-dichloro-a, a, a-trifluoro-p-tolyl)-4-trifluoro methyl sulfinyl pyrazole-3-carbonitrile (Tomlin, 2006).

FIP ($C_{12}H_4Cl_2F_6N_4OS$) is currently considered as the most reliable and popular chemical solution used to control pest that could cause significant damage to crops(McMahen *et al*., 2015; Pisa *et al*., 2015). FIP has been used to a variety of crops throughout the world, including rice, cotton, sorghum, maize, cereal grains (including wheat, barley, rye, and triticale), pasture grass, and straw. FIP due to its broad spectrum killing activity, can destroy the complete stages of insect's growth from larval to adult, including a variety of insects such cockroaches, mosquitoes, locusts, termites, rootworms, ticks, and fleas (Gunasekara *et al*., 2007).

Owing to excessive and uncontrolled use of these chemicals many pest insects had developed resistant to the available organophosphates, carbamates, and pyrethroid insecticides as a result of pesticide usage (Alyokhin *et al*., 2008; Simon-Delso *et al*., 2015). FIP is also very effective against insects that have resistance (Bobe *et al*., 1997; Gunasekara *et al*., 2007).

Different centers of human industry including agriculture the most specified one has been using fipronil more aggressively to control variety of pest. Owing to this aggressive and uncontrolled, non-specified used of fipronil causing damage to cell renewal, apoptosis and other DNA repair problems in insects and other beneficial insects of crops. Including to this, these pesticides are also responsible for hematopoietic and testicular tissues damages due to its toxicity and ability to cause oxidative stress (Hussain *et al*., 2014, Bhatti *et al*., 2016, Gul*et al*., 2017).

The present study was conducted on chickens. Experiment was performed on the age of 40 days chickens for 30 days. 80 samples were taken and divided into four groups. 20 samples were kept in each group. One group served as the control group and other three groups were used as experimental group different concentrations of fipronil were given to the experimental groups as 0.25mg/ml/kg. 0.50mg/ml/kg and 0.75mg/ml/kg different parameters as heamatology (as heamoglobin, HCV, MCV, MCHC, WBCs, RBCs, platelets), growth and oxidative stress (LPO and Catalase) were observed during this experiment. Growth was observed from first day, fifteen and at the end of experiment which was showing a decrease in growth as compared to control group. Levels of RBCs, WBCs, catalase and hemoglobin significantly decreased while the ALT, AST and LPO indicated a significant increase in their levels

In ourcurrent experimental study, we observed various clinico-physiological alterations done by these chemicals like fipronil such as depression, lowering of crowing, gasping, tremors and others as reduction in body weight and growth in birds was recorded. Due to the toxic effects of fipronil, different changes observed in behavior and clinics of insects and these changes may be due to effects of fipronil on the absorption of nutrients from the gut and also due to improper permeability of blood in blood vessels that could be the effects of fipronil which leads to anemia, fatigued activity and depression in insects (Ali *et al*., 2017).

In an earlier study, it has been reported that the considerable changes in blood parameters such changes in level of haemoglobin, RBCs, MCH, MTCH and others might be due poor efficiency of hematopoietic stem cells and also due to poor metabolic activities along with insufficient oxygen supply to blood forming tissues (Ghaffar *et al*., 2014).

A research was conducted to determine the impact of fipronil on several bird parameters. The results of the experiment demonstrated the time- and dose-dependent occurrence of clinical and behavioral disorders in birds. A 20, 40 and 60 days treatment of fipronil cause decline in RBCs and heamoglobin count. In cockerels (groups D and E) which are exposed high doses of FIP throughout the trial, hematocrit and total leukocyte count dramatically increased. Throughout the investigation, birds in groups D and E had significantly higher levels of cardiac isoenzyme (CK-MB), alanine transaminase and aspartate transaminase enzymes, serum urea and creatinine (kidney biomarkers), and liver function tests (Hussain *et al*., 2018). Similar to these findings, the current study also revealed an increase in urea, creatinine, ALT, and AST. Comparatively to the control group, clinical symptoms such anaemic comb, crowing, aggressiveness, and watery faeces were also seen in the experimental birds. In the current investigation, haemoglobin concentration also showed a decline.

Howeverresult of the present study revealed that oral administration of fipronil from high to even low doses not only induce clinical signs but also the hematological, growth and oxidative stress anomalies in chicken.

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Table 1: Growth estimation after introducing fipronil in *Gallus gallus domesticus*

Doses	**Initial weight**	**Weight after 15 days**	**Weight after 30 days**
Control group A	712.2±35.25	856.4^{a}±28.77	992.4^{a}±27.69
Group B (0.25mg/ml)	700.6±22.56	775.0^{ab}±23.51	867.9^{b}±24.59
Group C (0.5mg/ml)	730.8±12.36	772.8^{ab}±11.42	861.1^{b}±10.92
Group D (0.75mg/ml)	728.1±33.02	750.0^{c}±37.58	845.0^{b}±34.49

Table 2: Estimation of serum biochemical parameters in *Gallus gallus domesticus* after exposure to the different concentrations of fipronil

Enzyme	**Control group (A)**	**Group B (0.25mg/ml)**	**Group C(0.50mg/ml)**	**Group D(0.75mg/ml)**
ALT	1.738^{b}±0.143	2.513^{ab}±0.2467	2.600^{a}±0.1350	3.324^{a}±0.2873
AST	124.4^{b}±2.546	128.4^{ab}±3.057	132.8^{ab}±2.269	135.2^{a}±2.688
UREA	0.7038^{a}±0.044	0.7200^{a}±0.053	0.8200^{a}±0.089	0.8788^{a}±0.086
CREATININE	0.7125^{b}±0.061	1.163^{ab}±0.163	1.373^{a}±0.127	1.399^{a}±0.139

Table 3: Analysis of hematological parameters after introduction of fipronil in *Gallus gallu sdomesticus*

Hematological parameters	**Control group (A)**	**Group B (0.25mg/ml)**	**Group C(0.50mg/ml)**	**Group D(0.75mg/ml)**
Hb	10.48^{a}±0.424	8.933^{b}±0.380	8.322^{bc}±0.277	7.411^{c}±0.309
WBCs	124.1^{b}±6.389	145.6^{a}±5.654	154.8^{a}±2.212	161.6^{a}±1.483
RBCs	3.016^{a}±0.103	2.746^{ab}±0.092	2.526^{bc}±0.079	2.051^{c}±0.113
Platelets	14.56^{a}±2.572	19.78^{a}±3.670	25.78^{a}±3.031	24.89^{a}±3.306
MCV	137.8^{a}±1.512	135.0^{a}±1.500	136.6^{a}±1.459	138.1^{a}±1.455
MCH	40.89^{a}±0.388	41.56^{a}±0.765	41.33^{a}±0.645	42.22^{a}±0.547
MCHC	31.11^{a}±0.888	31.22^{a}±0.464	31.56^{a}±0.555	32.33^{a}±1.041
HCT	31.11^{a}±1.752	32.11^{a}±1.172	31.00^{a}±0.707	32.56^{a}±0.626

Table 4: Estimation of LPO and CAT level in the liver of *Gallus gallus domesticus* with respect to the different concentrations of fipronil

Enzymes		**Control group(A)**	**Group B(0.25mg/ml)**	**Group C(0.50mg/ml)**	**Group D(0.75mg/ml)**
LPO	Liver	2.784^{b}±0.063	2.862^{b}±0.158	3.124^{b}±0.098	4.593^{a}±0.205
	Kidney	2.106^{c}±0.277	2.935^{bc}±0.197	3.893^{b}±0.049	5.104^{a}±0.380
CAT	Liver	403.6^{a}±86.98	206.2^{ab}±22.26	177.1^{ab}±101.2	125.8^{b}±38.67
	Kidney	223.6^{a}±69.32	92.43^{ab}±42.04	95.33^{ab}±38.23	39.19^{b}±11.88

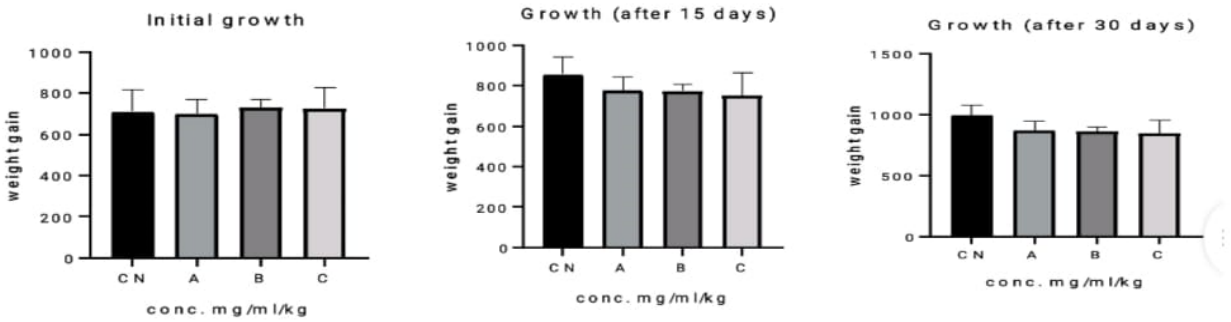

Fig.1: Growth estimation after introducing fipronil in *Gallus gallus domesticus*

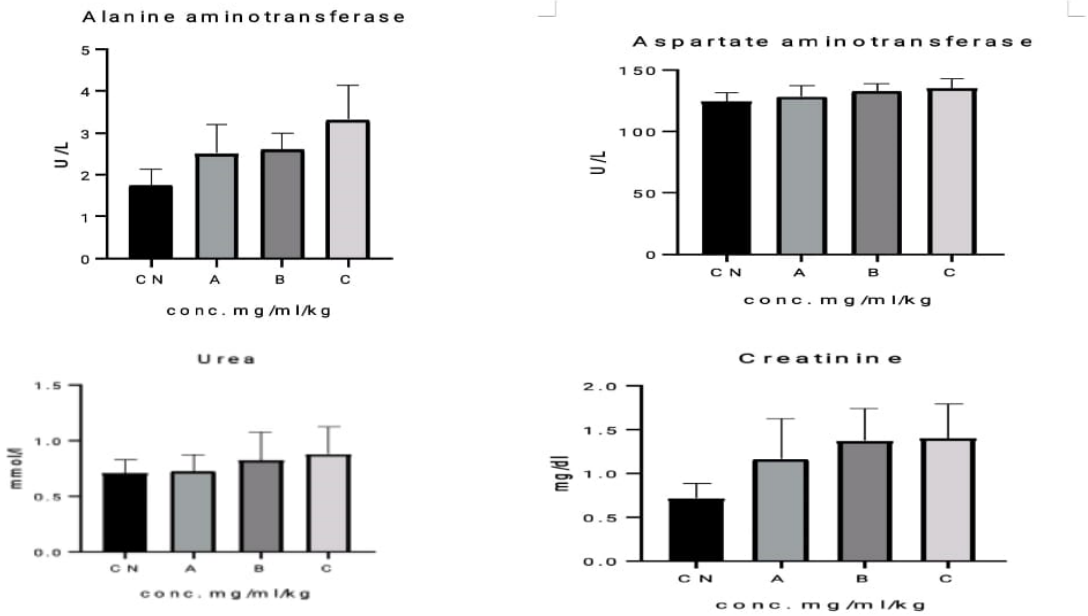

Fig.2: Estimation of serum biochemical parameters in *Gallus gallus domesticus* after exposure to the different concentrations of fipronil

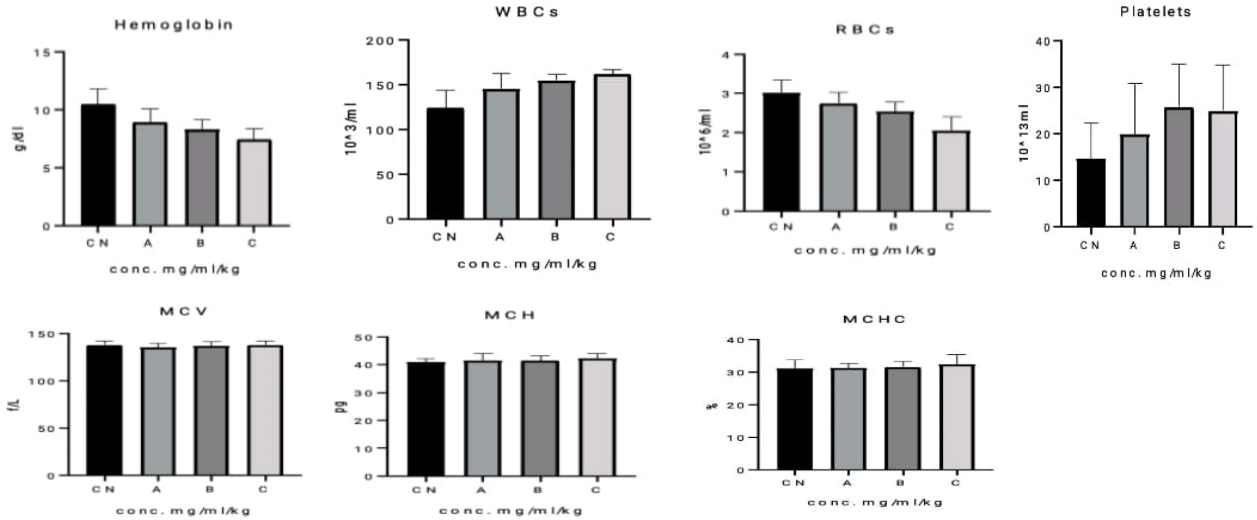

Fig.3: Analysis of hematological parameters after introduction of fipronil in *Gallus gallus domesticus*

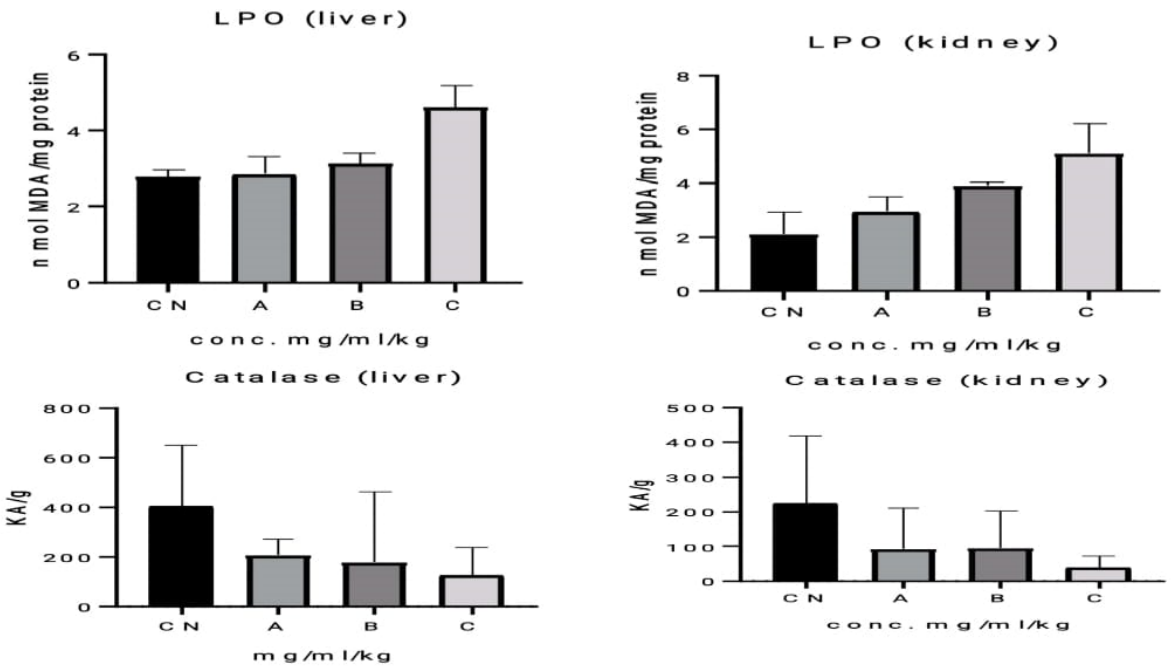

Fig.4: Estimation of LPO and CAT level in the liver of *Gallus gallus domesticus* with respect to the different concentrations of fipronil