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# Sub-acute Exposure of Fipronil Induces Biochemical and Histopathological Changes in the Liver, Kidney and Heart of Male Albino Rats

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## Abstract

Fipronil is chemically belong to the family of phenylpyrazole insecticides and has been used in agriculture and played a role in the health and food security. It has central nervous system effects and to date case reports are available on animal fatalities and human acute intoxications. The intoxication patterns were reported to be as ingestion and dermal exposure. The present study was designed to investigate the adverse effects of sub-acute exposure to the Fipronil, on the biochemical parameters, hematological parameters, and histopathological changes of male albino rats. Experimental animals were divided into four groups; group 1 was used as control, group 2, group 3, and group 4 were gavaged with: 7.5, 15, and 25 mg/kg body weight/day of Fipronil for 28 days, respectively. After two weeks from starting the experiment the blood values of the packed cell volume PCV and the mean corpuscular hemoglobin concentration MCHC were higher in group 4 than in the control ($p<0.05$). Furthermore, ALT and ALP serum activity and concentrations of total proteins albumin and globulin in groups 2 and 4 were higher than the control (group 1). Urea concentration in groups 3 and 4 was higher than in the other groups ($p<0.05$). After 4 weeks from starting the experiment there were no significant results in blood parameters. ALT and ALP serum activity and concentrations of total protein and globulin in groups 2 and 4 were higher than the control group (group 1). Albumin concentration in group 4 was lower ($p<0.05$) and urea concentration in groups 3 and 4 was higher ($p<0.05$) than the other groups. The concentration of cholesterol and creatinine did not change. The weekly gain of body weight was insignificantly changed between treatments to all groups. None of the animals died during the experiment period. Fipronil caused histopathological alterations in liver and kidney of male rats. From our results, it can be concluded that the pathological changes in liver, kidney and heart suggest a toxic effect of Fipronil to these tissues.

**Keywords:** Forensic toxicology; Fipronil; Rat; Sub-acute; Toxicity; Histopathological

## Introduction

Fipronil, 5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((1,R,S)-(trifluoromethyl) sulfinyl)-1-H-pyrazole-3-carbonitrile, is a phenyl pyrazole, broad-spectrum insecticide, first introduced to the US in 1996 for commercial turf and indoor pest control. It is particularly effective by way of ingestion and causes neural excitation and convulsions in insects, resulting in death [1,2]. Several case reports documented the human acute intoxication and animal fatalities that associated with the Fipronil ingestion and dermal exposure [3-5]. This issue has raised the risks of Fipronil against the health and food security. Fipronil is often used to control ants, beetles, cockroaches, fleas, ticks, termites, molecrickets, thrips, rootworms, weevils, and other insects [6]. It is more effective than organophosphate, carbamate and pyrethroids insecticides against several species of Lepidoptera, Orthoptera and Coleopteran [7,8]. The mode of action of Fipronil does not follow the common biochemical pathways of pyrethroids (sodium channel blockers), organophosphates, and carbamate (cholinesterase inhibitors), which are classical insecticides to which some insects have developed resistance [9]. However, Fipronil interferes with the γ-aminobutyric acid (GABA)-gated channels [10], and was reported to disrupts normal nerve influx transmission (e.g. passage of chloride ions) by targeting the GABA-gated chloride channel and at sufficient doses, causes excessive neural excitation, severe paralysis, and insect death [9,11,12]. The lower binding affinity for mammalian receptors enhances selectivity against insects and increases its boundary of safety [13]. Despite its obviously positive toxicological profile to mammals, Fipronil could have undesirable effects independent on non-neuronal sites like kidney, liver, and thyroid and influence reproductive function [14] in non-target organisms. Liver, kidney, and thyroid seemed to the most affected organs showing toxic effects on chronic exposure of Fipronil in rats [15]. Short-term exposure to Fipronil induced oxidative stress in the kidney, brain, and liver of mice [16]. Several researchers have expressed concern of probable undesirable human public health effects due to extensive and prolonged use of Fipronil in commercial and for home applications [17]. An important environmental concern of Fipronil exposure has been reported by Tingle et al. who stated that cyclic, systemic applications of Fipronil have bio accumulative effects, which in turn affect all animals along the trophic chain [18]. Hematological indicators are considered significant biomarker for suggesting altered internal and/or external environment of animals, and variations in their indicators within an individual may cause insufficient responses to chemical stressors; however, these variations are nonspecific to a wide scope of substances [19]. Hence, exposure to pesticides is supposed to increase or decrease the hematological levels and to frequently influence the survival of exposed organisms [20]. The biological markers including both hematological and biochemical parameters are exercised as good indicators of health conditions and outcome from the pesticide-induced toxicity within organisms [21]. For achieving food and health security, many of European countries and UK pulled millions of eggs from supermarket shelves after reporting the contamination of eggs with the potentially harmful insecticide Fipronil [22]. Therefore, the present study was performed in order to demonstrate the histopathological and biochemical alterations including liver function parameters; serum Aminotransferase (AST and ALT) and Alkaline Phosphates (ALP) activities, and kidney function parameters; serum urea and creatinine and hematological

parameters levels in male albino rats as affected by the daily orally administration of a single dose of Fipronil.

## Materials and Methods

### Animals and management

Twenty-four Wistar male rats aging twelve- week old and weighing between (220-260 g) were obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Animals were maintained on a 12 h light/dark in cycle polypropylene cages (six rats in each) at ambient temperature of 22 ± 1 °C and relative humidity of 50-60% with food and water provided ad libitum. All experiments were performed according to the recommendation of Experimental Animals Ethics and Rules of Naif Arab University for security sciences in accordance with the international standards for handling of experimental animals. The rats were acclimatized for 1 week before the start of the experiment.

### Chemicals and reagents

Fipronil (Insecto SC 5%) is a product of BASF Company and manufactured by, Bayer Co., Ltd., Germany. The assay kits used for biochemical measurements of aspartate Aminotransferases (AST, EC 2.6.1.1.), Alanine Aminotransferases (ALT, EC 2.6.1.2), Alkaline Phosphatase (ALP, EC 3.1.3.1), Total protein, albumin, Cholesterol, Urea and creatinine were purchased from United Diagnostics Industry, Dammam, and KSA.

### Experimental design

Sub-acute toxicity study (28-day repeated oral administration) was performed according to OECD 407 guidelines [23]. Rats were randomly divided into four experimental groups: group I received distilled water vehicle orally and served as a control group whereas groups II, III and IV received Fipronil at 7.5, 15 and 25 mg/kg of body weight, p.o., respectively (dissolved in distilled water). All rats were observed twice daily for mortality and morbidity till the completion of the experiment. They were observed for recording any clinical signs, the time of onset, duration of symptoms. Body weight of all rats was recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice. The concentrations of Fipronil were calculated depending on the percentage of active ingredients of commercial formulation of Fipronil. Concentrations of Fipronil were freshly prepared. Blood samples were collected from overnight fasted rats (only water allowed) after 2 and 4 weeks of treatment by retro orbital puncture into heparinized and non-heparinized tubes for hematological and biochemical analyses.

### Hematological parameters

The heparinized blood was used for the analysis of hematological parameters such as Hemoglobin (Hb) concentration, Red Blood Cells (RBCs) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and White Blood Cells (WBCs) count using (COULTER Ac•T 5 diff CP) Hematology analyzer.

### Biochemical parameters

The serum was separated from non-heparinized blood and the biochemical parameters including Aspartate Aminotransferases (AST), Alanine Aminotransferases (ALT), Alkaline Phosphatase (ALP), total protein, albumin, cholesterol, urea and creatinine were analyzed by using Semi-automatic biochemistry analyzer/compact BSA 3000 SFRI, French.

### Histology

After 2 and 4 weeks of treatment, 3 rats from each group were killed under diethyl ether anesthesia to identify gross lesions. Liver, heart, and kidney were collected from all the animals for histopathology. The collected organs were weighed and preserved in 10% neutral buffered formalin, sliced and finally a 5 µ thickness of tissue sections were stained with hematoxylin and eosin for histopathological study.

### Statistical analysis

Results were expressed as mean ± Standard Error Mean (SEM). Experimental data for statistics and correlations analysis were conducted by one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test. Graphing and data analysis were performed with SPSS version 16 statistical software (SPSS, Chicago, IL, USA) $p<0.05$ were considered statistically significant [24].

## Results

### Histopathological changes

After two weeks there were no significant or microscopic changes in rat tissue on the control group (group 1). Acute histopathological changes include liver necrosis, few lymphocytes infiltration, apoptosis, hepatocyte swelling, dilated sinusoids and central artery congestion observed in the liver. Congestion of glomeruli and tubules in the kidney were seen and there was congestion and hemorrhage in the heart. The rats exposed to a 15 mg/kg/bw dose of Fipronil (group 3) showed hepatocyte swelling, congestion and focal zonal necrosis and apoptosis in liver cells and focal necrosis in glomeruli and tubules of kidney. There was congestion in the heart too.

Figure 1 showed histopathological changes in the kidneys of rats exposed to 25 mg/kg/bw of Fipronil and the presence of eosinophilic material in tubules lumina and congestion.

Effects of Fipronil intake at different doses on growth of rats were shown in Table 1.

| Treatment groups | Initial body weight(g) | Body weight (g) | Body weight gain (g) |
|---|---|---|---|
| **2 weeks** | | | |
| Control (normal) | 254.6 ± 1.6$^{NS}$ | 299.1 ± 5.7$^{NS}$ | 44.5 ± 4.9$^{NS}$ |
| Fipronil (7.5 mg/kg/bw) | 262.5 ± 0.6$^{NS}$ | 300.8 ± 9.6$^{NS}$ | 38.3 ± 9.4$^{NS}$ |
| Fipronil (15 mg/kg /bw) | 267.3 ± 2.8$^{NS}$ | 290.8 ± 5.5$^{NS}$ | 23.5 ± 6.9$^{NS}$ |
| Fipronil | 277.5 ± 0.7$^{NS}$ | 306.8 ± 5.7$^{NS}$ | 29.3 ± 5.3$^{NS}$ |

| | | | |
|---|---|---|---|
| (25 mg/kg/bw) | | | |
| **4 weeks** | | | |
| Control (normal) | 321.6 ±1.2$^{NS}$ | 299.1 ± 5.7$^{NS}$ | 22.5 ± 4.5$^{NS}$ |
| Fipronil (7.5 mg/kg/bw) | 313.6 ± 19.2$^{NS}$ | 325.1 ± 13.8$^{NS}$ | 11.5 ± 5.4$^{NS}$ |
| Fipronil (15 mg/kg/bw) | 301.6 ± 2.18$^{NS}$ | 311 ± 2.2$^{NS}$ | 9.4 ± 0.02$^{NS}$ |
| Fipronil (25 mg/kg/bw) | 317.6 ± 4.3$^{NS}$ | 323 ± 4.4$^{NS}$ | 5.4 ± 0.1$^{NS}$ |

**Table 1:** Changes in average body weight and mean body weight gains in rats exposed different doses of Fipronil for 2 weeks and 4 weeks.

Values are means + SE; NS: Not Significant; *: $p<0.05$.

There was no significant change in the gained body weight weekly and none of the animals died during the experimental period.

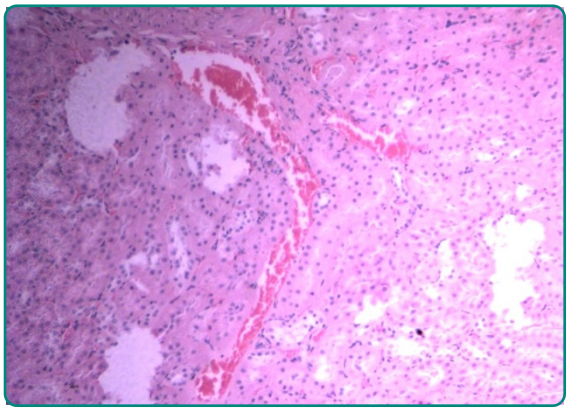

**Figure 1:** Eosinophilic material in tubules lumina and congestion in kidney rats exposed 25 mg/kg/bw/day of Fipronil for 15 days.

In the liver, focal zonal necrosis, apoptosis and inflammation of the biliary duct cells and dilated sinuses (Figure 2).

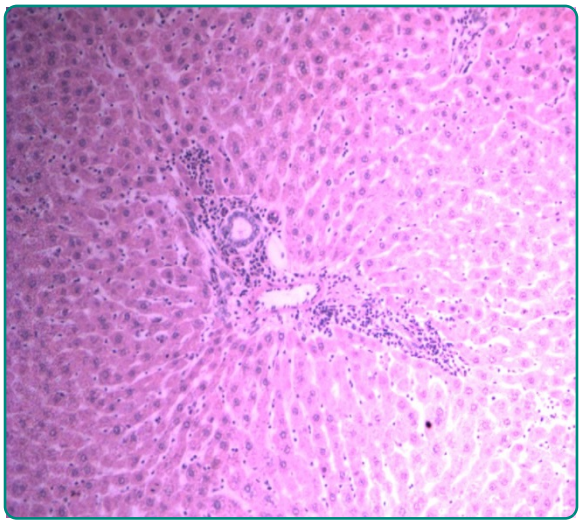

**Figure 2:** Chronic inflammatory cells infiltrate (lymphocytes) in portal tracts and sinusoids in liver rats exposed 25 mg/kg/bw/day of Fipronil for 15 days.

There was congestion and hemorrhage in the heart. At the end of the experiment, there was a hepatocyte swelling and inflammation of the biliary duct cells, granulocytes in the cellular fluid and dilated sinuses and necrosis in some liver cells (Figure 3).

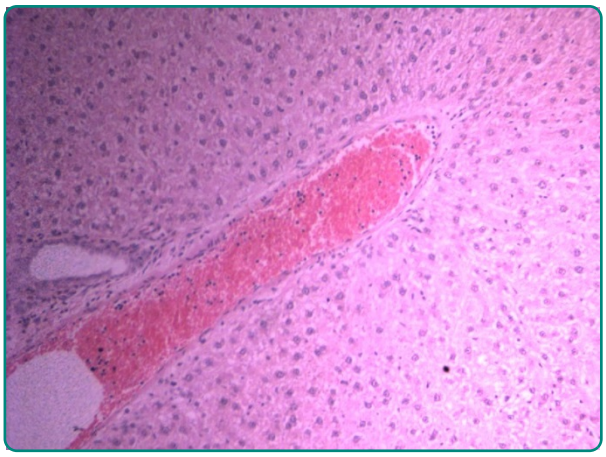

**Figure 3:** Hepatocytes swelling, chronic inflammatory cells infiltrated (eosinophils) in portal tract, granular cytoplasm, dilated sinusoid and zonal hepatocytes necrosis in liver rats exposed 7.5 mg/kg/bw/day of Fipronil for 30 days.

Congestion of glomeruli and tubules in the kidney and glomerular mesangial cells proliferation were observed. There was congestion in the heart of rats exposed to a dose of 7.5 mg/kg/bw Fipronil (group 2). Rats exposed to a 15 mg/kg/bw Fipronil showed hepatocytes granular cytoplasm dilated sinusoid (Figure 4), hepatocyte swelling and zonal hepatocytes necrosis, scattered apoptosis, and liver congestion. Tubular congestion in the kidney and heart congestion were seen. At the dose of 25 mg/kg/bw, focal tubular necrosis, eosinophilic and material in tubuler lumina (Figure 5) were shown in kidney.

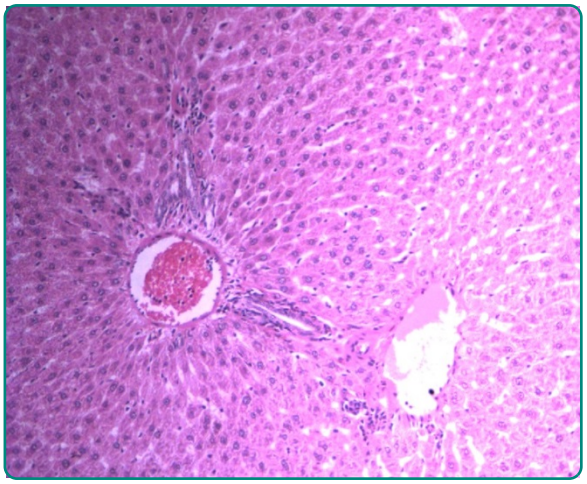

**Figure 4:** Few lymphocytes infiltrated in portal tract and dilated sinusoid in liver rats exposed 15 mg/kg/bw/day of Fipronil for 30 days.

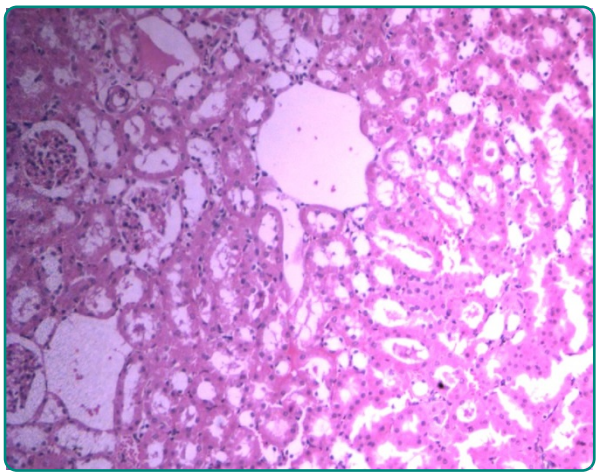

**Figure 5:** Focal tubular necrosis, esinophilic material in tuules lumina in kidney rats exposed 25 mg/kg/bw/day of Fipronil for 30 days.

In the liver, there were severe pathological changes including lymphocytic infiltration, dilated sinuses, hepatocyte swelling and zonal hepatocytes necrosis. Heart sections showed congestion.

## Hematological changes

After two weeks of treatment (Table 2), PCV and MCHC values in group 4 were higher ($p<0.05$) than in the control group and the values of Hb, MCH, MCV, WBCs, and RBCs have not changed. After 4 weeks of experiment, there was no change in the values of HB, PCV, MCH, MCV, MCHC, WBCs, and RBCs.

| Parameters | Control | Fipronil (7.5 mg\kg) | Fipronil (15 mg\kg) | Fipronil (25 mg\kg) |
|---|---|---|---|---|
| **2 weeks** | | | | |
| Hb (g/dL) | 15.4 ± 0.1 | 15.1 ± 0.2$^{NS}$ | 15.0 ± 0.7$^{NS}$ | 16.1 ± 0.4$^{NS}$ |
| WBCs($10^3$ $mm^3$) | 8.4 ± 1.2 | 12.2 ± 1.0 $^{NS}$ | 9.4 ± 1.9$^{NS}$ | 13.4 ± 1.4$^{NS}$ |
| PCV (%) | 50.9 ± 0.3 | 48.7 ± 0.4$^{NS}$ | 48.7 ± 1.8$^{NS}$ | 52.2 ± 1.2$^{*}$ |
| RBC ($10^6$ $mm^3$) | 7.8 ± 0.1 | 7.5 ± 0.0$^{NS}$ | 7.3 ± 0.2$^{NS}$ | 8.2 ± 0.2$^{NS}$ |
| MCV ($m^3$) | 64.7 ± 1.2 | 64.7 ± 0.3$^{NS}$ | 66.3 ±1.3$^{NS}$ | 63.3 ± 0.3$^{NS}$ |
| MCH (pg) | 19.8 ± 0.2 | 20.0 ± 0.1$^{NS}$ | 20.4 ± 0.3$^{NS}$ | 19.5 ± 0.1$^{NS}$ |
| MCHC (%) | 30.5 ± 0.3 | 31 ± 0.3$^{NS}$ | 30.8 ± 0.1$^{NS}$ | 30.8 ± 0.1$^{*}$ |
| **4 weeks** | | | | |
| Hb (g\dL) | 15.3 ± 0.1 | 15.5 ± 0.2$^{NS}$ | 15.9 ± 0.5$^{NS}$ | 14.8 ± 0.5$^{NS}$ |
| WBCs ($10^3$ $mm^3$) | 9.6 ± 2.5 | 10.4 ± 2.5$^{NS}$ | 12.3 ± 1.6$^{NS}$ | 12.3 ± 1.02$^{NS}$ |
| PCV (%) | 50.8 ± 0.7 | 49.6 ± 0.7$^{NS}$ | 51.7 ± 1.5$^{NS}$ | 48.3 ± 1.8$^{NS}$ |
| RBC ($10^6$ $mm^3$) | 7.7 ± 0.1 | 7.8 ± 0.2$^{NS}$ | 8.1 ± 0.3$^{NS}$ | 7.3 ± 0.2$^{NS}$ |
| MCV ($m^3$) | 66.0 ± 1.0 | 64.0 ± 0.6$^{NS}$ | 64.0 ± 0.6$^{NS}$ | 66.3 ± 1.3$^{NS}$ |
| MCH (pg) | 19.7 ± 0.2 | 19.9 ± 0.2$^{NS}$ | 19.8 ± 0.2$^{NS}$ | 20.4 ± 0.4$^{NS}$ |
| MCHC (%) | 30.4 ± 0.2 | 31.2 ± 0.2$^{NS}$ | 30.7 ± 0.01$^{NS}$ | 30.7 ± 0.01$^{NS}$ |

**Table 2:** Haematological changes in rats exposed to different doses of Fipronil for 2 weeks and 4 weeks.

Values are means + SE; NS: Not Significant; *: $p<0.05$.

## Sero biochemical changes

Two weeks after the start of the experiment (Table 3), ALT and ALP activities in groups 4-3-2 ($p<0.05$) were higher than in the control group and total protein concentrations, albumin and globulin in group 4 ($p<0.05$) were higher than in the control group (group 1). The concentration of urea in groups 3 and 4 was higher ($p<0.05$) than in other groups. There were no statistically significant changes in the concentration of cholesterol and creatinine. After 4 weeks of the experiment, ALT and ALP activities in groups 4,3,2 ($p<0.05$) were higher than in control group. Total protein concentrations and globulin in groups 4-3-2 ($p<0.05$) were higher than in the control group and albumin concentrations in group 4 was ($p<0.05$) lower than in other groups. Urea concentrations in groups 3 and 4 ($p<0.05$) were higher than in other groups. There were no statistically significant changes in the concentration of cholesterol and creatinine.

| Parameters | Control(normal diets) | Fipronil (7.5 mg \kg/bw) | Fipronil (15 mg \kg/bw) | Fipronil (25 mg \kg/bw) |
|---|---|---|---|---|
| **2 weeks** | | | | |
| ALT(iu) | 76.4 ± 0.26 | 94.8 ± 2.41$^{*}$ | 92.90 ± 2.90$^{*}$ | 350.57 ± 43.30$^{*}$ |
| ALP(iu) | 115.67 ± 4.63 | 282.67 ± 45.83$^{*}$ | 294.0 ± 26.69$^{*}$ | 307.67 ± 37.56$^{*}$ |
| T.protien(g/dL) | 5.33 ± 0.33 | 8.0 ± 0.58$^{*}$ | 7.67 ± 0.67$^{*}$ | 8.0 ± 0.58$^{*}$ |
| Albumin(g/dL) | 3.0 ± 0.0 | 3.33 ± 0.33$^{*}$ | 3.67 ± 0.33$^{*}$ | 3.33 ± 0.33$^{*}$ |
| Globulin(g/dL) | 2.33 ± 0.33 | 4.67 ± 0.67$^{*}$ | 4.0 ± 0.58$^{*}$ | 4.67 ± 0.33$^{*}$ |
| Cholestrol(mg/dL) | 56.10 ± 2.30 | 94.09 ± 3.35$^{NS}$ | 95.96 ± 0.42$^{NS}$ | 100.19 ± 6.70$^{NS}$ |
| Urea(mg/dL) | 28.39 ± 5.06 | 54.30 ± 2.76$^{NS}$ | 66.98 ± 1.03$^{*}$ | 63.88 ± 1.41$^{*}$ |
| Creatinine(mg/dL) | 0.43 ± 0.03 | 0.67 ± 0.09$^{NS}$ | 0.67 ± 0.03$^{NS}$ | 0.73 ± 0.07$^{NS}$ |
| **4 weeks** | | | | |
| ALT(iu) | 59.80 ± 3.05 | 91.63 ± 0.73$^{*}$ | 108.17 ± 0.41$^{*}$ | 350.57 ± 43.30$^{*}$ |

| | | | | |
|---|---|---|---|---|
| ALP(iu) | 110.0 ± 5.57 | 300.0 ± 56.03* | 156.33 ± 0.88* | 274.67 ± 17.17* |
| T.Protien(g/dL) | 6.0 ± 0.0 | 7.67 ± 0.33* | 7.67 ± 0.33* | 8.0 ± 0.58* |
| Albumin(g/dL) | 3.67 ± 0.33 | 4.67 ± 0.33NS | 4.67 ± 0.33NS | 3.33 ± 0.33* |
| Globulin(g/dL) | 2.33 ± 0.33 | 3.0 ± 0.0* | 3.0 ± 0.0* | 4.67 ± 0.33* |
| Cholestrol(mg/dL) | 58.56 ± 3.62 | 89.77 ± 4.82NS | 90.32 ± 2.60NS | 100.19 ± 6.70NS |
| Urea(mg/dL) | 26.03 ± 1.65 | 52.19 ± 1.46NS | 57.95 ± 0.44* | 62.88 ± 0.53* |
| Creatinine(mg/dL) | 0.33 ± 0.03 | 0.57 ± 0.03NS | 0.77 ± 0.09NS | 0.73 ± 0.07NS |

**Table 3:** Serobiochemical changes in rats exposed to different doses of Fipronil for 2 weeks and 4 weeks.

Values are means ± SE; NS: Not Significant; *: $p<0.05$.

## Discussion

There was no difference in the average body weight among the different groups along the experiment and this may be explained by that all animals received the same food supply. While the clinical signs associated with the doses of Fipronil (groups 3 and 4) included a change in activity and abnormal walking observed from the third week, the possibility of Fipronil to induce specific neurotoxicity in mice [25], rats [26] and man [27] was possible. The present results showed that the sub-acute exposure to different doses of Fipronil caused liver and kidney damage, as indicated in the biochemical parameters Transaminases (ALT and AST), which plays an important role in the destruction and biosynthesis of amino acids. They are responsible for detoxification, metabolism and bio-synthesis of biomolecules of various essential functions and used as specific indicators of liver damage [28,29]. The increase in these enzymes may be assigned to a liver dysfunction and possible disruption in the biosynthesis of these enzymes with an alteration in the possibility of liver membrane permeability [30]. The toxicity data of Fipronil formulation (FIP) showed that the LD50 value obtained after 24 h was 143.50 mg AI/kg/bw in albino mice [31]. Ola et al. found that taking oral Fipronil at a dose of 0.5 mg/kg/day for 21 days resulted in an increase in the activity of LDH, AST, acid phosphatase, gamma-glutamyl transferase and concentrations of total protein and glucose in buffalo calves [32]. These results were supported by other studies, which demonstrated that the increase in transaminases and total protein concentration was associated with liver cell damage [33]. The increase in urea concentration in the current study is a sign of impaired renal function. This is consistent with the findings of El-Demerdash and Yousef et al., that urea is the final product of protein destruction that is considered as an indication of renal dysfunction [34,35]. Therefore, Urea, uric acid and plasma creatinine levels are the parameters of kidney function [36]. Tingle et al. showed that thyroid gland, liver, and kidneys of rat affected by chronic exposure to Fipronil [18]. Histopathological observations on liver and kidneys of Fipronil-exposed rats were comparable with other studies conducted on chlorpyrifos, Fenitrothion and other pesticides [37-39]. These studies showed that these insecticides raise few pathological parameters in the liver and kidneys. In this study, changes in the liver and kidney biomarkers combined with tissue damage in Fipronil-exposed rats were observed. Histopathological hepatocellular observations showed severe zonal necrosis, lymphocytic infiltration, dilated sinuses, hepatocyte swelling, scattering apoptosis, and hepatic congestion. The kidneys have severe necrosis, inflammation, and focal tubular necrosis, eosinophilic material in tubules lumina, Glomerular ascites congestion, and focal tubular congestion. These observations indicated clear changes in the general condition of the liver and kidneys in response to Fipronil. These changes can be the result of the toxic effects of Fipronil primarily by the generation of reactive oxygen species that cause damage to many components of the cell membrane. The observations in these studies are additional proof and support for our findings. It is clear from the results of the current study that the liver and kidneys are sensitive organs to the toxic action of the insecticide used Fipronil.

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