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IMPACT OF FIPRONIL ON THE LIVER METABOLISM OF SWISS MALE ALBINO MICE

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ABSTRACT

The main objective of the present study is to detect the effect of fipronil on the liver of Swiss albino mice. There are very few reports about the effect of fipronil exposure on the mice liver during adults, in the present study 9.5mg/kg body weight, 95mg/kg body weight fipronil was treated by oral gavage to mice. Swiss albino mice were divided into three groups each consisting of six animals i.e. control, group-1, and group-2. The activity levels of Aspartate aminotransferase (AAT), Alanine transaminase (ALT), Succinate dehydrogenase (SDH), and Lactate dehydrogenase (LDH) were significantly ($p \leq 0.05$) increased in the liver of all experimental mice compared to control mice. The activity levels of Lipid peroxidation, SOD and Catalase were also significantly ($P \leq 0.05$) increased in all Fipronil treated mice compared to control mice liver tissue. From the results of the present study, it is suggested that exposure to fipronil might cause dysfunction of the liver which leads to affect the glucose metabolism and activity levels of antioxidants in animals and humans.

1. INTRODUCTION

Now a day's Pesticides are abundantly used worldwide in agricultural non-agricultural practices to control pests and increase crop yield. India is the largest manufacturing country of pesticides in Asia and ranks 12th on the global spectrum (Indira et al., 2007). During the past few decades, there is a gradual increase in the utilization of pesticides in both agriculture and non- agricultural areas in the form of sprays, poisons, and powders for controlling cockroaches, mosquitoes, rats, ticks, and other harmful bugs (WHO, 1990). According to previous studies, 5.2 billion pounds of pesticides are used worldwide per year, which affects human and animal health. These pesticides are commonly organochlorides,

organophosphorus, carbamates, pyrethroid, and different organic compounds. Testes, liver, kidney, and brains are the most sensitive and principal target organs of organophosphate pesticide toxicity and injury (Mansour et al., 2010; Akhila and Sreenivasula Reddy., 2018).

Fipronil is one the type of organophosphate (Phenyl pyrazole chemical family) pesticide used as an insecticide to control ants, ticks, rootworms, lice and ticks in dogs, cats and cattle by disrupting the normal function of the central nervous system (Chaton et al., 2002; Aajoud et al., 2003). Fipronil can commonly penetrate the food chain from the crops and diet is the main source of this pesticide exposure to humans and animal's bodies. After ingestion into the body, the higher concentration was found mainly in fatty tissue and break down into smaller metabolites in humans and rats, causes cancer. Some of the previous studies related to fipronil toxicity are, long term exposure to fipronil, oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) were significantly reduced, while lipid peroxidation was significantly increased in treated rats liver and kidney (Das et al., 2006). According to the study of Oliveira et al., 2012, reported that rats are exposed to the different dosage levels of fipronil like 15, 25, and 50 mg/kg body weight which affects the cytological and immunological changes and necrotic cell death in their liver.

Although a large number of studies have been reported on adverse effects of organophosphorus pesticides on liver metabolisms, there have been fewer reports dealing particularly with the toxicity of fipronil on liver metabolisms.

2. MATERIALS AND METHODS

2.1. Animal model

Male albino Swiss albino mice weighing between 30g to 35g and age of 3 weeks were obtained from Sri Venkateswara Enterprises, Bangalore, used for the present experimental study. Mice were acclimatized for 10 days to the animal house in polypropylene ages with top stainless steel grill and maintained under standard photoperiodic condition and temperature and feed with standard pellet diet and provide water ad libitum.

2.2. Chemicals

Bovine serum albumin (BSA), nicotinamide adenine dinucleotide (NAD) was purchased from Sigma chemical company, St. Louis, Missouri, USA. Fipronil ($C_{12}H_4Cl_2F_6N_4OS$, CAS No: 120068-37-3) was purchased from Bhagiradha chemicals and private Limited,

Telangana, India. All other chemicals used for the present study were technical grade and obtained from local commercial sources.

2.3. Experimental design

Healthy female Swiss albino mice (body weight 35±2 g) were selected for the present study. They were reared in an air-conditioned animal house facility (23±2 ˚C; light; dark; 12:12 hrs; relative humidity (55±5%) at the Department of Zoology, SV. University, Tirupati, India. The mice (n= 24) were maintained in polypropylene cages lined with sterilized paddy husk as a bed. The mice were provided with a standard pellet diet (purchased from Bangalore, India). Mice were randomly divided into 3 groups, each group consisting of 8 animals. Group-1 served as control, Group-2 received 9.5mg/kg body weight of fipronil (1/10 of LD50), and Group-3 animals treated with 95mg/kg body weight (LD50) of fipronil respectively by oral gavage. Dosage levels are selected for the present study was based on the study of Tomlin, 2006. The treatment continued for 10 days daily and all the animals were maintained for another 25 days. All experimental group animals were allowed to grow on a normal diet, and the bodyweight was recorded for every 5 days. During the experimental period, mice were observed daily for overall appearance and toxicity. After 25 days the mice were sacrificed by cervical dislocation. The liver was isolated immediately and transfers into Petri dish containing normal saline (0.9% NaCl). The liver was cleared from adhering tissues/fluids, blotted on filter paper, and weighed to their nearest milligram by using Shimadzu electric balance. Liver tissue somatic indices (TSI) were calculated by using the formula: [Weight of the tissue (g)/body weight of the animal (g)]/ 100. All experiments were carried out in accordance with the guidelines and protocol (Regd. No. 438/01/a/CPCSEA/dt.17/07.2001), Sri Venkateswara University, Tirupati.

2.4. Determination of lipid peroxidation

The levels of lipid peroxidation in the liver of mice were measured in terms of malondialdehyde (MDA; a product of lipid peroxidation) content and determined by using the thiobarbituric acid (TBA) reagent. The reactivity of TBA is determined with minor modifications of the method adopted by Hiroshi et al. (1979).

2.5. Assay of anti-oxidant enzyme activities

Catalase (E.C:1.11.1.6) activity in the liver of mice was determined by the method of Chance and Machly, 1955). The catalase activity was expressed as µmoles of H2O2 metabolized /mg protein / h. SOD (E.C.1.15.1.1) activity was determined in the liver by the method described

earlier (Misra and Fridovich, 1972). At alkaline pH, Superoxide anion O2- causes the antioxidation of epinephrine to adrenochrome, while completing this reaction, SOD decreased the adrenochrome formation. One unit of SOD is defined as the amount of extract that inhibits the rate of adrenochrome formation by 50%. The activity of SOD was expressed in units/mg protein/min.

2.6. Estimation of energy metabolisms

The Lactate dehydrogenase in the liver of control and experimental mice was estimated by the method of Srikarrthan and Krishna Murthy, (1955) in the liver of control and experimental mice. The enzyme activity was expressed as µm of formazan formed/mg protein/hr.

The Succinate dehydrogenase activity in the liver of mice was estimated by the method of Nachals et al., 1960. The enzyme activity was expressed as µm of formazan formed/mg protein/hr.

The Alanine aminotransferase and Aspartate aminotransferase activity were assayed in the liver tissue of mice by the method of Reitman and Frankel (1957) and the enzyme activity was expressed as µm of pyruvate formed/ mg protein/hr.

2.7. Statistical analysis

The data were statistically analyzed by using a one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test using the statistical package for the Social Sciences 16 version. The data were presented as mean ± S.D. Differences were considered to be significant at $p<0.05$.

3. RESULTS AND DISCUSSION

The effect of organophosphorus insecticides on animals shows adverse effects on major organs like liver, kidney and also the nervous system, immune system and reproductive system (Kossmann et al., 1997; Nagymajtennyi et al., 1998; Gomes et al., 1999; Aly and El-gendy, 2000; Mansour and Mossa, 2011). The liver is the principal organ for actively participate in carbohydrates, lipids, proteins, minerals, and vitamin metabolism and is responsible for the metabolism of xenobiotics (detoxification). Aspartate aminotransferases play an important role in amino acid biosynthesis and detoxification.

Some of the previous studies related to fipronil toxicity on the liver were prolonged exposure of fipronil in different concentrations like 0.1, 1 and 10 mg/L in drinking water for 45 days may increase alanine transaminase and aspartate transaminase in male rats (Mossa et al., 2015), inhibited the oxygen uptake and increased the cytosolic NADH/NAD+ ratio under glycolytic conditions. The effects on oxygen uptake indicated that that possible mechanism of toxicity of fipronil involves impairment on mitochondrial respiratory activity and therefore, interference with energy metabolism and increased lipid peroxidation in liver and increased alanine aminotransferase in liver cells (Awad et al., 1998, Tukhtaev et al., 2013, Medeiro et al., 2015).

In the present study body weights and liver (Table 1) indices were not changed significantly in all experimental rats compared to control rats indicating the general health of rats was not altered. In the present results activity levels of superoxide dismutase and catalase were decreased significantly (Figure 2 and 3) in all experimental rats compared to control might be due to increased the concentration of malondialdehyde, these results are in agreement with earlier reports Das et al., 2006; Alijani Ardeshir et al., 2017. Increased oxidative stress and lipid peroxidation (Figure 1) are might be due to pesticides induced toxicity (Khehrer, 1993).

Exposure of cells to oxygen free radicals may cause lipid peroxidation in cell membranes which in turn may generate radical species that damage cell proteins and promote their degradation. Lipid peroxidation levels are increased in the liver by exposure to fipronil. Activity levels of Catalase (Figure 3) and Superoxide dismutase (Figure 2) were increased significantly ($P < 0.05$) in liver tissue of all experimental mice compared to control mice indicating its active involvement in the decomposition of peroxide radicals and hydrogen peroxide thereby decreasing the toxicity. To understand the energy-related alter actions in glycolysis and Kreb's cycle, the SDH activity is decreased and LDH activity levels were increased in liver tissue (Table 1). In the present study decrease in SDH activity indicates the reduced oxidative metabolism of mitochondria is due to the fipronil effect leading to the hydroxyl group-containing enzymes and mitochondrial swelling. The increased levels of LDH and decreased levels of SDH confirm a shift in the normal balance of glycolysis in favor of anaerobiosis. GDH, AAT, ALT enzymes activity levels were increased in the liver of all experimental mice. The probable explanation for this behavior is mainly an adaptive mechanism of the animal to meet the energy demand, against the fipronil to maintain the metabolic homeostasis. In the present study energy metabolites (AAT, LDH, and ALT) are

increased significantly in all experimental rats compared to control. Aspartate aminotransferases play an important role in amino acid biosynthesis, detoxification, and increase energy metabolites indicate liver dysfunction and disturbance s in the biosynthesis of these enzymes.

Table 1: Effect of Fipronil in the Liver of mice.

Parameters	Control	Group-1	Group-2
Body weights	33.36±2.46	32.47^{a}±1.82 (-2.67%)	34.13^{a}±2.56 (2.31%)
Liver index	5.22±0.66	5.00^{a}±0.67 (-4.21%)	5.37^{a}±0.63 (2.87%)
Lactate dehydrogenase (µg of formazan formed/mg protein/hr)	0.91±0.21	2.37^{b}±0.18 (160.43%)	2.53^{c}±0.18 (178.02%)
Glutamate dehydrogenase (µg of formazan formed/mg protein/hr)	1.39±0.12	2.77^{b}±0.64 (99.28%)	4.52^{c}±0.25 (225.89%)
Aspartate amino transferase (µg of pyruvate formed/mg protein/hr)	1.36±0.01	4.44^{b}±1.43 (226.47%)	5.2^{b}±0.34 (282.35%)
Alanine amino transferase (µg of pyruvate formed/mg protein/hr)	0.41±0.11	1.61^{b}±0.25 (292.68%)	2.27^{c}±0.08 (453.65)
Succinate dehydrogenase (µg of formazan formed/mg protein/hr)	3.9±0.41	2.45^{b}±0.51 (-37.18%)	2.75^{b}±0.24 (-29.49%)

Values are mean ± S.D.

Values in the parentheses are percent change from that of control.

Values are significantly different at $p<0.05$. a = Not significant, b, c = Significant.

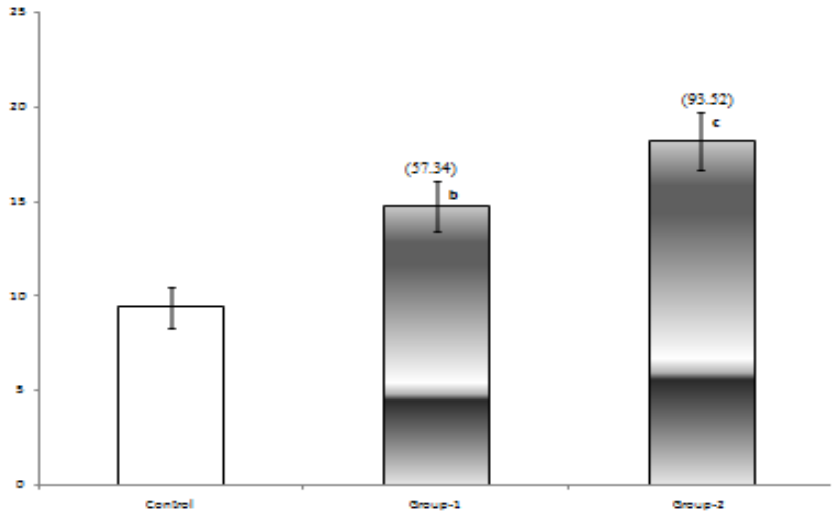

Figure 1: Effect of Fipronil on lipid peroxidation in the liver of mice.

Bars are mean ± S.D of 6 individuals

Bars that do not share same superscript differ significantly from each other at p< 0.05

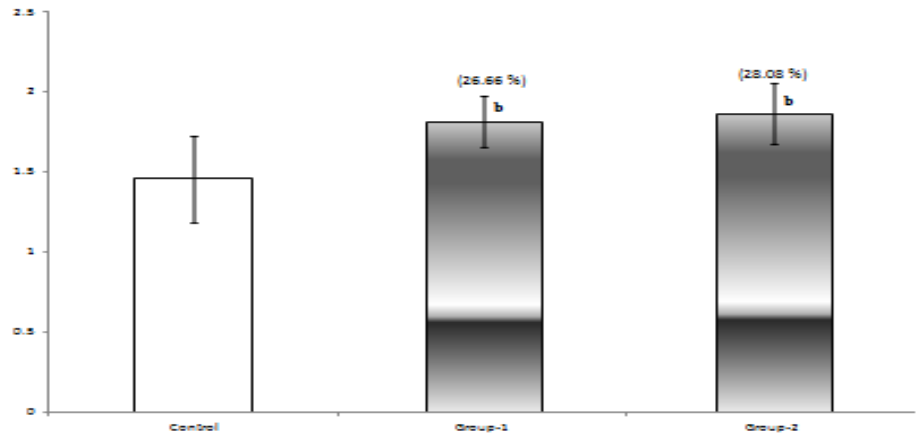

Figure 2: Effect of Fipronil on Superoxide dismutase in the liver of mice.

Bars are mean ± S.D of 6 individuals

Bars that do not share same superscript differ significantly from each other at p< 0.05

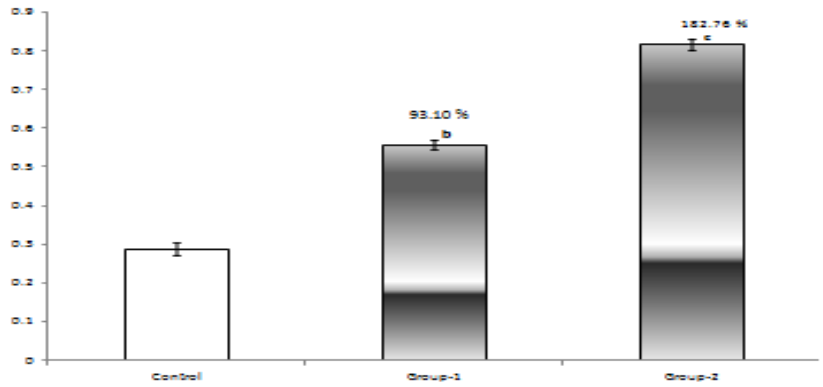

Figure 3: Effect of Fipronil on Catalase activity in the liver of mice.

Bars are mean ± S.D of 6 individuals

Bars that do not share same superscript differ significantly from each other at p< 0.05

5. CONCLUSION

The results of the present study bring out the exposure to fipronil during adults in group-1 and group-2 is a responsible increase in lipid peroxidation, LDH, AAT, ALA, and antioxidants like SOD, catalase significantly whereas SDH was decreased significantly in all experimental animals compared to controls. In conclusion, this study provides compelling evidence of decreased SDH and increased energy metabolizes SOD and Catalase. The maximum elevation of oxidative stress and energy metabolism was observed in group-2 experimental mice compared to control mice might be due to the toxic effect of fipronil. These changes are potentially harmful and affect the metabolism of mice liver.

6. ACKNOWLEDGEMENT

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7. Declaration of Conflicting Interests

The authors declare that there are no conflicts of interest that would prejudice the impartiality of this scientific work.

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