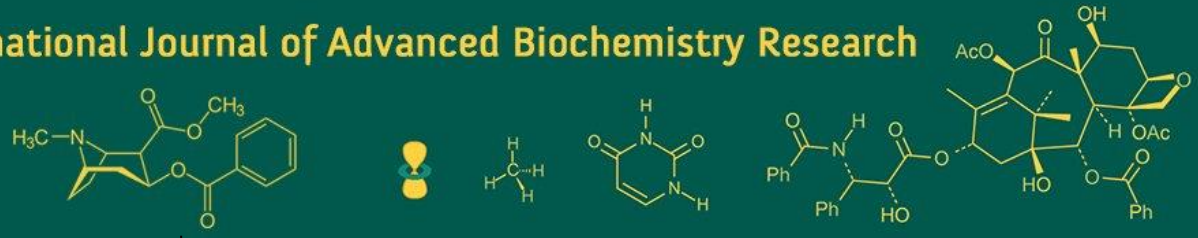
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(1): 330-333
www.biochemjournal.com
Received: 01-10-2023
Accepted: 08-11-2023

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Assessment of Fipronil toxicity following subacute dermal exposure in rats

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DOI: https://doi.org/10.33545/26174693.2024.v8.i1e.372

Abstract
Fipronil is a phenyl pyrazole, broad-spectrum insecticide, used for control of pests in veterinary and agriculture sector. Present study was designed to evaluate the toxicity of fipronil following daily dermal application in Albino rats for 28 days. Rats were divided in to three groups having six animals in each group. Group I was treated as control and rats in group II and III were dermally applied with fipronil at dose 100 and 200 mg.kg^{-1} b.wt. Respectively daily for 28 days. Hematological and biochemical parameters were determined at 0, 14th and 28th day of exposure. The present investigation resulted in significant elevation in aspartate amino transaminase, alanine amino transaminase, alkaline phosphatase, lactate dehydrogenase activities in fipronil treated rats at both dose levels, indicated liver damage Fipronil also induced kidney damage as evidenced by significant increase in blood urea nitrogen and creatinine level. Also, the effect of fipronil on biochemical parameters was dose dependent. However, no significant changes on hematological parameters were observed at both the doses of fipronil during exposure period. Present study suggests hepatic and renal toxicity potential of fipronil following repeated dermal exposure.

Keywords: Fipronil, dermal application, hematological parameters, biochemical parameters, rats

Introduction
A pesticide is any chemical that is used to control, eradicate, or repel a particular species of plant or animal that is considered a pest. Horticulture, forests, gardens, homes, and businesses all utilize insecticides. They exist in both biological and chemical forms. Insecticides indirectly aid in the growth of the agriculture and livestock sectors by controlling pests. As a result, using them is essential and cannot be avoided, but doing so effectively is just as important. Misuse, improper handling, or prolonged exposure to them may be detrimental. Due to their numerous applications and related effects, pesticides have gained significant attention from scientist Apart from enhancing agricultural output and assisting with pest control initiatives, it has led to the emergence of certain health problems. Fipronil, a broad-spectrum phenyl pyrazole insecticide, was first introduced to the US market in 1996 for use in commercial grass management and indoor pest control. Fipronil binds noncompetitively to GABAA-gated chloride channels in the central nervous system, hence inhibiting the inhibitory effect of GABA. Insects are killed by this, which results in neural hyperexcitation and paralysis. (Guelfi *et al*., 2015 and Gupta, 2018) [7, 8]. It is used to control ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, thrips, rootworms, weevils, and other insects. It is reported to be effective even against those pests which have gained resistance to the conventional insecticides (Kartheek and David, 2018) [11]. Because fipronil is widely used in the veterinary, agricultural, and home sectors, there is a growing risk of contamination of food, water, and air as well as exposure to people, domestic animals, and the environment, all of which can have negative consequences. (Swelam *et al.*, 2017) [15].
Pesticide exposure can happen orally, by inhalation or through the skin, and it can have harmful consequences on aquatic, animal, and human health. Additionally, those who work in the pesticide manufacturing industry are exposed at work place. In work environments, dermal exposure to pesticides and their internal absorption are seen as serious issues. Hematological and biochemical markers are among the biological markers that are thought to be reliable predictors of health outcomes and diseases resulting from pesticide-induced toxicity (Abdelgadir *et al*., 2020) [3].

Fipronil has been shown in numerous oral toxicity studies to cause hematological, biochemical, oxidative stress, and histopathological changes in experimental animals exposed to the drug over an extended period of time (Mossa *et al*., 2015, Karthik and David, 2016, 1017) [13, 9-10]. However, repeated dermal exposure to fipronil has not yet been studied. Thus, the purpose of the current study was to find out the possible dermal toxicity of fipronil in rats during long term exposure.

Materials and Methods

Experimental animals

Eighteen healthy, well-fed male Wistar albino rats, weighing between 150 and 200 grams, were used in the experiment. They were kept in normal temperature and humidity conditions for two weeks prior to the start of the study so they could become acquainted to their new environment. Animal rooms were maintained at 23±2 °C with a light/dark photoperiod of 12:12 hours and a relative humidity of 45.0 (±15) %. The animals were fed a standard pellet meal and provided water *ad libitum*. The rats were divided into three groups, each consisting of six rats.

The Institutional Animal Ethics Committee (IAEC) of the institute has approved all protocols and standard guidelines for the maintenance of experimental rats that are followed, as per the guidelines of CPCSEA.

Dose and administration

Acute dermal LD_{50} of fipronil is more than 2000 $mg.kg^{-1}$ b.wt. in rats. (U.S. EPA, 2003). So, the $1/10^{th}$ dose of 2000 $mg.kg^{-1}$ b.wt. (200 $mg.kg^{-1}$ b.wt.) and $1/20^{th}$ dose of 2000 $mg.kg^{-1}$ b.wt. (100 $mg.kg^{-1}$ b.wt.) were decided to choose for sub-acute dermal toxicity study of fipronil.

About a day before the test, the fur of animals was removed by shaving the dorsal part of their trunks, which extended the lateral midline on both sides, from the scapulae to the ilium wing. This region makes up about 10% of the body's surface. Subsequently, the animals had weekly shavings without experiencing any skin damage. Using a porous gauze bandage fastened with non-irritating tape, the test substance was kept in contact with the skin for the duration of the 28-day study period. The test substance was held in contact with the skin with a porous gauze dressing covered with non-irritating tape to retain the gauze for at least 6 hrs. per day exposure throughout the study period of 28 days.

Collection of blood samples

Blood samples from rats of each group were collected at 0, 14^{th} and 28^{th} day of study from medial canthusus with the help of 1ml tuberculin syringe in clean and dry vial for estimation of hematological parameters, which was coated with anticoagulant (EDTA), another vial without anticoagulant for estimation of biochemical parameters.

For hematology, about 1 ml of blood was drawn into a sterile vial containing 2 ml of EDTA per ml of blood. The remaining 1 ml of blood was drawn into a centrifuge tube devoid of anticoagulant for serum separation. Following blood clotting, the vial was centrifuged for five minutes at 2000 rpm, collecting serum in a sterile vial that was stored at -20 °C for biochemical analysis.

Hematological parameters

All the hematological parameters were determined by method as described by Wills, (2010) [18].

Serum biochemical parameters

Liver function biomarkers like, Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydogenase (LDH) and kidney function biomarkers like, blood urea nitrogen (BUN) and creatinine were estimated as per the method of Teitz (1999) [16].

Experimental Design

The animals after acclimatization to the laboratory conditions, randomly divided into seven groups (6 rats in each group) and classified as follow:

Group I (Normal control group): Rats applied with no drugs.

Group II (Fipronil treated group): Rats dermally exposed with fipronil at dose 100 $mg.kg^{-1}$ b.wt. daily for 28 days.

Group III (Fipronil treated group): Rat dermally exposed with fipronil at dose 200 $mg.kg^{-1}$ b.wt. daily for 28 days.

Statistical analysis

Data were expressed as mean ± SE. The data were subjected to one-way analysis of variance and further subjected to Tukey's test for post hoc analysis by defining the significance level at $p<0.05$. All statistical analyses were performed using SPSS software (Version 20.0).

Results and Discussion

No any morality was recorded in rats under control and exposed groups. Moreover, dermal application of fipronil did not induce any prominent signs toxic signs in exposed rats at both dose levels. Loss of body weight was observed in both the fipronil treated groups at different intervals (Table 1). The reduction in body weight of rats may be due to oxidative stress induced by fipronil (Mossa and Abbassy, 2012) [12].

No significant changes in hematological parameters were found at both doses of fipronil. Similar results were reported by Abdelgadir *et al*., 2020 [3] in a 28 days exposure of 25 $mg.kg^{-1}$ b.wt. of fipronil in rats.

Fipronil caused significant increase ($p<0.05$) in the activities of AST, ALT, ALP and LDH at both dose level on 14^{th} and 28^{th} day of exposure compare to '0' day (Table 2, 3, 4, 5). When compared with control group on 28^{th} day, at both dose level of fipronil, there was 64.29 and 92.2% increase in AST activity, 47.69 and 57.5% increase in ALT activity, 61 and 69.37% increase in ALP level, 18.98 and 27.7% increase in LDH level, respectively. The results indicated dose dependent effect of fipronil. In different studies also, fipronil induced significant ($p<0.05$) increase in AST, ALT, ALP and LDH activities (Mossa *et al*., 2015; Al-Harbi, 2016; Hussain *et al*., 2018; Abdel-Daim and Abdeen, 2018; Abdelgadir *et al*., 2020 and Yadav *et al*., 2020) [3, 13, 5, 6, 2, 3, 19].

Since AST, ALT, ALP, and LDH are involved in detoxification processes, metabolism, and the synthesis of energy macromolecules for numerous essential functions, they are specifically used as markers for liver damage. Changes in the permeability of the liver membrane, liver illness, and production disturbances can all lead to an overexpression of these enzymes. Hepatocelluar necrosis may result in increased LDH activity and bloodstream enzyme leakage (Mossa *et al*., 2015 and Al-Harbi, 2016) [13, 5]. Cytoplasmic marker enzyme (ALP) assays are a useful

tool for predicting the prognosis of liver and lung illnesses, since they serve as an indicator of the damage that hazardous chemicals cause to cells and tissues.

Level of BUN was significantly (p<0.05) increased at 14th and 28th day compare to '0' day level on exposure of both doses of fipronil. When compared with control group on 28th day there was 48.1 and 76.54% increase in BUN level in group I and II respectively (Table 6). Fipronil also significantly (p<0.05) increased the creatinine level at dose 100mg/Kgbwt. on 28th day compare to '0' day level whereas, creatinine level changed significantly (p<0.05) on 14th and 28th day of exposure of fipronil at dose 200 mg/Kgbw compare to '0' day. When compared with control group on 28th day, creatinine level was increased by 74.22 and 94.84% for both doses (Table 7) The results revealed that fipronil induced kidney damage, as evidenced from significant (p<0.05) elevation of BUN and creatinine level and the effects were dose dependent. Significant (p<0.05) increase in BUN and creatinine level due to fironil exposure in separate studies was also reported (Abdel-Daim and Abdeen, 2018 and Yadav *et al.,* 2020) [2, 19].

An increase in protein catabolism in the mammalian body is connected with elevated blood urea levels. (Abbassy and Mossa, 2012) [12]. A metabolite of creatine, creatinine is entirely eliminated through glomerular filtration in the urine. Therefore, an increase in its blood level indicates compromised renal function (Mossa and Abbassy, 2012) [12].

Table 1: Effect of repeated 28-days dermal exposure of fipronil on body weight (g) of rats

Groups	0 day	7th day	14th day	21st day	28th day
I	141.73 ± 3.43^{b}	143.80 ± 2.86^{b}	146.68 ± 2.81^{ab}	149.71 ± 3.24^{ab}	152.66 ± 3.37^{a}
II	140.81 ± 2.78^{a}	137.28 ± 2.77^{ab}	134.88 ± 2.93^{ab}	133.51 ± 3.04^{ab}	131.88 ± 3.13^{b}
III	153.03 ± 4.56^{a}	149.28 ± 4.16^{ab}	145.16 ± 4.25^{b}	140.21 ± 5.09^{bc}	137.01 ± 4.98^{c}

Table 2: Effect of repeated 28-days dermal application of fipronil on Aspartate aminotransferase activity (IU/L) in rats of different groups

Groups	0 day	14th day	28th day	Percent increase
I	52.53 ± 1.98^{a}	50.38±2.58^{a}	50.66 ± 1.14^{a}	-
II	51.76 ± 3.02^{c}	65.93±2.91^{b}	83.23 ± 2.43^{a}	64.29
III	52.70 ± 1.85^{c}	71.23±0.87^{b}	97.40 ± 3.54^{a}	92.2

Table 3: Effect of repeated 28-days dermal application of fipronil on Alanine aminotransferase activity (IU/L) in rats of different groups

Groups	0 day (Mean ± S.E)	14th day (Mean ± S.E)	28th day (Mean ± S.E)	Percent increase
I	37.65 ± 1.24^{a}	38.48 ± 1.53^{a}	38.31 ± 0.97^{a}	-
II	37.28 ± 2.88^{c}	46.68 ± 2.14^{b}	56.58 ± 1.46^{a}	47.69
III	38.48 ± 1.00^{c}	46.00 ± 1.25^{b}	60.35 ± 2.05^{a}	57.5

Table 4: Effect of repeated 28-days dermal application of fipronil on Alkaline phosphatase (IU/L) in rats of different groups

Groups	0 day	14th day	28th day	Percent increase
I	48.93 ± 1.95^{a}	50.16 ± 1.21^{a}	51.65 ± 2.49^{a}	-
II	48.10 ± 1.86^{c}	64.30 ± 1.42^{b}	83.23 ± 3.01^{a}	61
III	47.13 ± 1.48^{c}	62.51 ± 1.98^{b}	87.48 ± 4.62^{a}	69.37

Table 5: Effect of repeated 28-days dermal application of fipronil on Lactate dehydrogenase (IU/L) in rats of different groups

Groups	0 day	14th day	28th day	Percent increase
I	214.08 ± 1.78^{a}	215.05 ± 2.40^{a}	211.46 ± 1.44^{a}	-
II	213.16 ± 2.17^{c}	221.36 ± 3.20^{b}	251.60 ± 1.70^{a}	18.98
III	212.55 ± 3.08^{c}	230.93 ± 2.42^{b}	270.03 ± 3.11^{a}	27.7

Table 6: Effect of repeated 28-days dermal application of fipronil on Blood urea nitrogen (mg/dl) in rats of different groups

Groups	0 day	14th day	28th day	Percent increase
I	35.83 ± 1.19^{a}	35.81 ± 1.31^{a}	36.03 ± 1.11^{a}	-
II	37.91 ± 1.47^{c}	45.10 ± 1.86^{b}	53.38 ± 0.67^{a}	48.1
III	37.06 ± 1.18^{c}	48.08 ± 2.24^{b}	63.61 ± 2.25^{a}	76.54

Table 7: Effect of repeated 28-days dermal application of fipronil on Creatinine (mg/dl) in rats of different groups

Groups	0 day	14th day	28th day	Percent increase
I	1.03 ± 0.05^{a}	1.08 ± 0.06^{a}	0.97 ± 0.02^{a}	-
II	1.12 ± 0.05^{b}	1.22 ± 0.04^{b}	1.69 ± 0.12^{a}	74.22
III	0.99 ± 0.01^{c}	1.28 ± 0.03^{b}	1.89 ± 0.13^{a}	94.84

Conclusion

Based on overall findings from the our study, it may be inferred that fipronil is toxic to liver and kidney following repeated dermal exposure at selected doses, as it is known to alter the biochemical levels and the effects were found to be dose dependent. These data might be used for risk assessment. This study could be concluded by further cautioning the farmers about harmful effects of fipronil upon its long term exposure. Therefore, a great care is to be taken during application of fipronil in agriculture and veterinary sectors.

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