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Durgesh Yadav
Department of Pharmacology and Toxicology, College Veterinary Science and A.H., Mhow, Indore, Madhya Pradesh, India

Gayatri Dewangan
Department of Pharmacology and Toxicology, College Veterinary Science and A.H., Mhow, Indore, Madhya Pradesh, India

Neetu Rajput
Department of Pharmacology and Toxicology, College Veterinary Science and A.H., Mhow, Indore, Madhya Pradesh, India

Corresponding Author:
Gayatri Dewangan
Department of Pharmacology and Toxicology, College Veterinary Science and A.H., Mhow, Indore, Madhya Pradesh, India

Ameliorating effect of quercetin against fipronil induced subacute toxicity in rats

Durgesh Yadav, Gayatri Dewangan and Neetu Rajput

Abstract
Fipronil is a class of phenyl pyrazole pesticides used for the control of a wide range of agricultural, public health and veterinary pests. Quercetin, the flavonoid present in several vegetables and fruits possesses a number of pharmacological activities. Present study was conducted to evaluate the ameliorative effect of quercetin against fipronil induced toxicity in rats. Rats were divided in to three groups, having six animals in each group. Group I was treated as control and group II and III received, fipronil (10 mg/kg b.wt) and fipronil + quercetin (10mg/kg b.wt +100mg/kg b.wt) respectively orally daily for 28 days. Blood samples for estimation of biochemical parameters were collected on 0, 14 and 28th days of exposure. The results revealed that fipronil caused significant reduction in body weight of rats during 28 days of exposure which may be due to oxidative stress by induced by fipronil. Fipronil also significantly increased the serum level of AST, ALT, ALP LDH, BUN and creatinine during 28 days of exposure indicated liver and kidney damage by fipronil. However supplementation of quercetin mitigated the adverse effects of fipronil by reducing the elevated level of serum biochemical parameters.

Keywords: Fipronil, quercetin, biochemical parameters, protective effect, rats

Introduction
Pesticides are heterogeneous group of substances used for preventing, destroying or repelling pests. Animals are infested by a number of parasitic insects and acarine species causing major economic losses in agriculture and livestock industry. Growing demand of pesticides and their indiscriminate application has led to environmental contamination globally, causing adverse health effects on non-target organisms. Fipronil is a class of phenyl pyrazole pesticides used for the control of a wide range of agricultural, public health and veterinary pests (Swelam *et al.*, 2017) [18]. It has been found to be effective even against those pests which have gained resistance to the conventional insecticides (Kartheek and David, 2018) [13]. Fipronil is an active ingredient of one of the popular ectoparasiticide veterinary products, Frontline, commonly used on dogs and cats to kill fleas and all stages of ticks and mites (Gupta, 2018) [8]. Because fipronil is widely used in agriculture, veterinary sector and household applications, the high rates of possible contamination (e.g., food, water and air) and exposure (e.g., human, domestic animals and environment) are increasing. Therefore, recent concerns for potential adverse public health effects of fipronil have been raised (Swelam *et al.*, 2017) [18].
The toxic action of fipronil is due to its ability to act on gama aminobutyric acid receptor. Fipronil binds noncompetitively to $GABA_A$-gated chloride channels, thereby blocking the inhibitory action of GABA in the central nervous system, which lead to the death of insect by neuronal hyperexitation and paralysis (Guelfi *et al.*, 2015; Gupta, 2018) [7, 8]. Fipronil induces hematological, biochemical, oxidative stress and histopathological changes (Kartheek and David, 2016, 2017; Mossa *et al.*, 2015) [11, 12, 16] during long-term exposure in rats.
Quercetin, the flavonoid present in several vegetables and fruits also possesses antioxidant and other biological activities including antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, protective of the reproductive system and anti-obesity agent (Maalik *et al.*, 2014; Aluani *et al.*, 2016 and Sharma *et al.*, 2018) [15, 4, 17]. There has been report of protective effect of vitamin E and vitamin C (Badgujar *et al.*, 2014) [6] zinc, (Swelam *et al.*, 2017) [18] rosuvastatin (Abdel-Daim and Abdeen, 2018) [1], Ginseng (Al-Harbi, 2016) [3] against fipronil induced toxicity. Quercetin has been found to effective against toxicity induced by pesticides of other groups like neonicotinoids, organophosphate and pyrethroides (Li *et al.*, 2016, Auwal and Kumar, 2017 and sharma *et al.*, 2018) [5, 17]. However there are no report regarding protective effect of quercetin against fipronil induced toxicity.

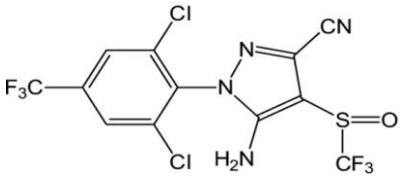
Material and Methods

Experimental animals

Eighteen male Wistar albino rats (6-8 weeks) weighing 150-200gm were obtained from animal house of Veterinary college Mhow, Indore (M.P.) The animals were housed in a room maintained under a 12/12 h light– dark cycle, an ambient temperature of 23-30 °C with a relative humidity of 45 (±15)%. Animals were provided with food with free access standard pellet diet and water *ad libitum*.. All rats were housed for a duration of 1 week for acclimatization before initiation of the experiments. The maintenance of experimental rats and all the procedures implemented are in accordance with standard guidelines issued by CPCSEA followed with approval of the Institutional Animal Ethics Committee (IAEC) of the institute.

Chemicals

Fipronil (Trade name frontline) was obtained from local market and quercetin from S.V. agro foods limited, Mumbai (Maharashtra) India (U.S. EPA, 2009) [20]



Chemical structure of fipronil

Dose and administration

Fipronil was administered orally at dose rate of 10mg.kg^{-1}b.wt (1/10th of $LD_{50,}$ U.S EPA, 2009) and quercetin at 100 mg.kg^{-1}b.wt of each for 28 days.

Collection of blood samples

Blood samples were collected from rats of different groups at 0, 14th and 28th day of study from tail vein with the help of 1ml tuberculine syringe About 1ml blood was collected in sterile vial containing anticoagulant EDTA @ 2mg/ml of blood for haematology and remaining 1ml of blood was collected in a centrifuge tube without anticoagulant for serum separation. After clotting of blood the vial was centrifuged @ 2000 rpm for 5 minutes and serum was collected in a sterile vial and was preserved at -20ºC for biochemical estimation.

Serum biochemical parameters

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydogenase (LDH), blood urea nitrogen (BUN) and creatinine were determined by method described by Teitz (1999) [19].

Experimental design

After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (6 rats each) placed in individual cages and classified as follow:

Group I (**normal control group**): Rats received no drugs, served as control.

Group II (**Fipronil exposed group**): Rats received fipronil at dose rate of 10 mg.kg^{-1}b.wt. Orally daily for 28 days.

Group III (Fipronil+Quercetin treated group): Rat received fipronil at dose rate 10 mg.kg^{-1}b.wt. and quercetin at 100 mg.kg^{-1}b.wt. Orally daily for 28 days.

Statistical analysis

Data were reported 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

Signs of toxicity

No mortality or signs of toxicity were recorded in rats of group I, II and III during study period. Kartheek and David (2018) [13] also observed no mortality or severe clinical sings of toxicity in rats given fipronil @ 32.33, 12.12 and 6.46 mg kg^{-1}b.wt. for 90 days.

Effect on body weight

Body weight of animal in control group increased significantly ($P<0.05$) at weekly intervals whereas, in group II, body weight was significantly ($P<0.05$) reduced at 7th, 14th, 21th, and 28th day compare to '0' day values. On the other hand in group III variation in body weight was nonsignificant at different weeks compare to '0' day values (Table 1).

Table 1: Mean values of Body weight (g) of in rats of different groups (n=6) at weekly interval

Groups.	0 day	7th day	14th day	21st day	28th day
I	151.83±0.65^{d}	155.33±0.71^{cd}	159.17±0.40^{bc}	163.67±0.40^{ab}	166.50±0.60^{a}
II	153.33±0.88^{a}	141.67±1.82^{b}	139.17±2.50^{bc}	134.67±2.30^{bc}	133.50±2.10^{c}
III	154.00±1.43^{a}	150.33±1.42^{a}	151.66±1.33^{a}	152.66±1.11^{a}	153.13±1.07^{a}

Mean values bearing different superscripts within rows (within groups) differed significantly ($P<0.05$)

Effect on biochemical parameters

AST and ALT

AST and ALT level was significantly ($P<0.05$) increased in group III on 28th day compare to '0' day. But, value of ALT and AST differed nonsignificantly at 14th day, compared to '0' day value. When compared with control group on 28th day, AST level increased by 110.18 % and by 65.32 % increase in group II. and group III respectively and ALT was increased by, 69.7 in group II and by 22.18 % in group III.

ALP

Level of ALP was significantly ($P<0.05$) increased in group II at 14th and 28th day of exposure compare to '0' day. Similarily in group III significant ($P<0.05$) increase in ALP level was recorded at 14th and 28th day of exposure compare to '0' day. When compared with control group on 28th day, ALP activity was increased by 156.42, and 94.5 in group II. And III respectively.

LDH

Significant ($P<0.05$) increase in LDH level was found in group II and III at 14th and 28th day of exposure compared to '0' day values but when compared with control group on 28th day, increase in LDH level was 55.59, and 25.22 % respectively in group II and III.

BUN

Level of BUN was significantly (P<0.05) increased at 14th and 28th day compare to '0' day level in group II. A significant (P<0.05) variation in level of BUN was also noted in III on 28th day, compare to 0 day value. When compared with control group on 28th day, there were 105.71 and 64.21% increase in BUN level in group II and III respectively.

Creatinine

Level of creatinine was significantly (P<0.05) increased in group II, on 14th and 28th day compared to '0' day level but creatinine level changed significantly in group III at 28th day of exposure, compare to '0' day. When compared with control group on 28th day, the percent increase in creatinine level in group II, III was 99.01 and 53.92 respectively.

Treatment with fipronil caused significant increase in the level of ALT, AST, LDH, ALP, BUN and creatinine after 28th day of exposure as compared to control group. Also in group treated with fipronil plus quercetin, the level of biochemical parameters was significantly increased during 28 days exposure, however, the values were much lower than the fipronil treated group (Table 2, 3, 4, 5, 6 and 7).

Table 2: Mean values of Aspartate aminotransferase (IU/L) at 0, 14th and 28th day of study in rats of different groups (n=6)

Group	0 day	14th day	28th day	Percent increase
I	47.57±2.05 [a]	48.50±2.79 [a]	49.45±2.57 [a]	-
II	52.62±1.70[c]	81.74±4.53 [b]	104.33±3.64[a]	110.98
III	68.64±3.35 [b]	70.80±1.98 [b]	81.95±2.57 [a]	65.32

Mean values bearing different superscripts within rows (within groups) differed significantly (P<0.05)

Table 3: Mean values of Alanine aminotransferase (IU/L) at 0, 14th and 28th day of study in rats of different groups (n=6)

Groups	0 day	14th day	28th day	Percent increase
I	40.95±3.04 [a]	43.17±1.99 [a]	42.15±2.18 [a]	-
II	47.14±1.97 [c]	64.43±1.91 [b]	71.57±1.58 [a]	69.70
III	33.01±3.46 [b]	39.01±3.11[b]	51.50±2.56 [a]	22.18

Mean values bearing different superscripts within rows (within groups) differed significantly (P<0.05)

Table 4: Mean values of Alkaline phosphatase (IU/L) at 0, 14th and 28th day of study in rats of different groups (n=6)

Groups	0 day	14th day	28th day	Percent increase
I	45.16±2.09 [a]	46.03±1.28 [a]	45.53±1.82 [a]	-
II	56.54±0.83[c]	81.15±2.16[b]	116.28±0.84[a]	156.42
III	54.71±1.18 [c]	76.82±2.17[b]	88.56±1.96[a]	94.50

Mean values bearing different superscripts within rows (within groups) differed significantly (P<0.05)

Table 5: Mean values of Lactate dehydrogenase (IU/L) at 0, 14th and 28th day of study in rats of different groups (n=6)

Groups	0 day	14th day	28th day	Percent increase
I	219.33±1.80 [a]	220.33±1.02 [a]	220.66±1.47 [a]	-
II	211.83±1.72 [c]	288.66±2.67 [b]	343.33±2.16 [a]	55.59
III	215.50±2.09 [c]	237.50±1.66 [b]	276.33±1.80 [a]	25.22

Mean values bearing different superscripts within rows (within groups) differed significantly (P<0.05)

Table 6: Mean values of Blood urea nitrogen (mg/dl) at 0, 14th and 28th day of study in rats of different groups (n=6)

Groups	0 day	14th day	28th day	Percent increase
I	38.16±2.26 [a]	41.58±2.71 [a]	33.95±4.22 [a]	-
II	31.05±1.65 [c]	54.62±3.17[b]	69.78± 1.79 [a]	105.71
III	41.61±2.63 [b]	43.72±0.58 [b]	55.75±2.09 [a]	64.21

Mean values bearing different superscripts within rows (within groups) differed significantly (P<0.05)

Table 7: Mean values of Creatinine (mg/dl) at 0, 14th and 28th day of study in rats of different groups (n=6)

Groups	0 day	14th day	28th day	Percent increase
I	1.31±0.02 [a]	1.13±0.23 [a]	1.02±0.05 [a]	-
II	1.22±0.07 [b]	1.45±0.05 [b]	2.03±0.04 [a]	99.01
III	1.13±0.09 [b]	1.12±0.06 [b]	1.57±0.06 [a]	53.92

Mean values bearing different superscripts within rows (within groups) differed significantly (P<0.05)

Discussion

Increase in body weight of rats in control group indicates normal weight gain of rats but in fipronil group, the body weight of rats was decreased at weekly interval during 28 days of treatment which may be due to oxidative stress induced by fipronil. On the other hand supplimentation of quercetin, attenuated the fipronil induced toxicity. Body weight of rats did not change significantly, when administered with fipronil at dose rate 2mg kg^{-1}b.wt, orally for 45 days (Mossa *et al.*, 2015; Swelam *et al*., 2017) [16, 18]. The variation in results of present study may be due to higher dose of fipronil.

The results of the study indicated that fipronil induced liver and kidney damage in treated rats at dose rate 10 mg/ kg^{-1}b.wt. when administered daily for 28 days, as shown by significant increases in serum marker enzymes AST, ALT, ALP, LDH,BUN and creatinine Our findings are in accordance with the previous studies (Mossa *et al*., 2015; Al-Harbi, 2016; Hussain *et al*., 2017; Abdel-Dam and Abdeen, 2018) [16, 3, 9, 1].

AST, ALT, ALP and LDH are mainly used in the evaluation of hepatic damage. Transaminases (AST and ALT) play an important role in amino acids catabolism and biosynthesis. They are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential functions and used as specific indicators for liver damage. The increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of the liver membrane takes place. The elevation in LDH activity may be due to the hepatocelluar necrosis and leakage of the enzyme into the blood (Mossa *et al*., 2015 and Al-Harbi, 2016) [16, 3].

Assay of ALP can be used for the prognosis of liver and lung disorder. ALP, cytoplasmic marker enzyme, is a known indicator of cell and tissue damage by toxic compounds. (Al-Ananay *et al.*, 2015).

Quercetin is a phytochemical with proven antioxidant and cyto-protective activities. (Sharma *et al.*, 2018) [17]. Co-administration of quercetin decrease the elevated level of AST, ALT, ALP, LDH indicating their protective effect against fipronil induced toxicity. Hussein *et al*, (2017) [10] and Al-Ananay *et al*., (2015) also reported that quercetin produced ameliorative effect against metalaxyl fungicide and nicotine induced liver toxicity, respectively in male rats.

Supplementation of quercetin may also reduce the renal damage by inducing decline in BUN and creatinine level. Protective effect of quercetin against organophophate and thiamethoxam insecticide induced nephrotoxicity in rats was reported by Li *et al.* (2016) and Auwal and Kumar (2017) [5].

Conclusion

From the present study it can be concluded that fipronil induced liver and kidney damage, as evidenced by changes in liver and kidney function biomarkers, when administered at dose rate of 10 $mg.kg^{-1}$b.wt. Orally for 28 days. Co-

administration of quercetin ameliorated the toxic effects of fipronil by reducing the elevated level of biochemical parameters. The finding suggest that quercetin produced protective effect against fipronil induced subacute toxicity by mitigating the adverse effects of fipronil.

References

1. Abdel-Daim MM, Abdeen A. Protective effects of rosuvastatin and vitamin E against fipronil mediated oxidative damage and apoptosis in rat liver and kidney. Food and Chemical Toxicology 2018;114:69-77.
2. Al-Anany MGE, Kamal AM, Saied KE. Effects of curcumin and/or quercetin on nicotine-induced lung and liver toxicity in adult male albino rat. Al-azhar Assiut Medical Journal 2015;13(2):93-103.
3. Al-Harbi MS. Fipronil Induced hepatotoxicity, genotoxicity, oxidative stress and the possible ameliorative effect of ginseng. British Journal of Pharmaceutical Research 2016;14(5):1-14.
4. Aluani D, Tzankova V, Yordanov Y, Zhelyazkova A, Georgieva E, Yoncheva K. Quercetin: an overview of biological effects and recent development of drug delivery systems. Pharmacia 2016;63:52-60.
5. Auwal MS, Kumar V. Effect of subchronic oral exposure of male rats to thiamethoxam, quercetin and their combination on oxidative stress parameters in kidney. BAOJ Veterinary Science 2017;1(1):2.
6. Badgujar PC, Pawar NN, Telang AG, Kurade N, Chandratre GA, Kadave M. Histopathological alterations induced by subacute fipronil toxicity in mice and its amelioration by combination of α-tocopherol and ascorbic acid. Indian Journal of Veterinary Pathology 2014;38(1):29-32.
7. Guelfi M, Maioli MA, Tavares MA, Mingatto FE. Citotoxicity of fipronil on hepatocytes isolated from rat and effects of its biotransformation. Brazilian Archieves of Biology and Technology 2015;58(6):843-853.
8. Gupta RC. Veterinary Toxicology: Basic and Clinical Principles, 3rd Edition, Acadmic press, Cambridge, USA 2018, 533-538.
9. Hussain R, Ghaffar A, Ali HM, Abbas RZ, Khan JA, Khan IA. Analysis of different toxic impacts of fipronil on growth, hemato-biochemistry, protoplasm and reproduction in adult cockerels. Toxin Reveiwes 2018:37(4):294-303.
10. Hussein SA, El-sinosi AY, Mansour MK, Hassan MF. Potential protective effects of Quercetin on metalaxyl-induced oxidative stress impaired liver functions and hepatotoxicity in rat. Benha Veterinary Medical Journal 2017;33(2):517-532.
11. Kartheek RM, David M. Fipronil induced modulations in biochemical and histological aspects of male wistar albino rats: A subchronic study. World Journal of Environmental Biosciences 2016;5(2):26-32.
12. Kartheek RM, David M. Assessment of renal toxicity in rats exposed to commercial formulations of fipronil. International journal of pharmaceutical, chemical and biological science 2017;7(3):303-310.
13. Kartheek RM, David M. Assessment of fipronil toxicity in wistar rats: A hepatotoxic prospective. Toxicology reports 2018;5:448-456.
14. Li S, Cao C, Shi H, Yang S, Qi L, Zhao X. Effect of quercetin against mixture of four organophosphate pesticides induced nephrotoxicity in rats. Xenobiotica, 2016;46(3):225-33.
15. Maalik A, Khan FA, Mumtaz A, Mehmood A, Azhar S, Atif M *et al*. pharmacological applications of quercetin and its derivatives: a short review. Tropical Journal of Pharmaceutical Research 2014;13(9):1561-1566.
16. Mossa ATH, Swelam ES, Mohafrash SMM. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. Toxicology Reports, 2015;2:775-784.
17. Sharma P, Khan IA, Singh R. Curcumin and quercetin ameliorated cypermethrin and deltamethrin-induced reproductive system impairment in male wistar rats by upregulating the activity of pituitary-gonadal hormones and steroidogenic enzymes. International Journal of Fertility and Sterility 2018;12(1):72-80.
18. Swelam ES, Abdullah IS, Mossa ATH. Amelorating effect of zinc against oxidative stress and lipid peroxidation induced by fipronil in male rats. Journal of pharmacology and toxicology 2017;12(1):24-32.
19. Teitz NW. Textbook of clinical chemistry. Publ. W.B. Saunders Co. Philadelphia 1999, 582-584.
20. US. EPA (United states Environmental Protection Agency). EPA's records disposition schedule pest 361 scientific data reviews health effects division file R177146. Office of prevention pesticides and toxic substances. United States Environmental Protection Agency Washington D.C 2009, 1-39.