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Impact of Fipronil Sulfone on Oxidative Stress Biomarkers and Thyroid Hormones in Male Albino Rats

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ABSTRACT

The current 28-day repeated dose oral toxicity study in rats was carried out to assess the toxicity of fipronil sulfone. Male albino rats (4 groups of 5 animals) were administered fipronil sulfone via gavage at doses of 0.01, 0.1 and 1.0 mg/kg bw/day. The levels of both total protein and creatinine significantly increased after fipronil sulfone treatment in a dose-dependent manner as compared to control. Moreover, the activity of aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were highly significant increased compared to the control group. Highly significant changes in all oxidative stress biomarkers; superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione peroxidase (GPx), glutathione reduced (GSH) and lipid peroxidation (LPO) were observed also in liver tissues at the treatment of 1.0 mg/kg bw/day.Fipronil sulfone treatments were also associated with an increase in serum thyroid-stimulating hormone (TSH) levels after 28 days at all tested doses. The triiodothyronine (T3) and thyroxine (T4) serum concentrations were 49 and 43% lower in fipronil sulfone treated rats, respectively at 1.0 mg/kg as compared to untreated rats after 28 days of treatment. This highlights the need to further investigate the contribution of fipronil sulfone to the fipronil-induced thyroid disruption.These results also show promise for detailed analyses of these biomarkers and their linkages to biological pathways.

Keywords: Fipronil Sulfone, Oxidative Stress, Biomarkers, Hormones, Rat

INTRODUCTION

Our world undergoes rapid changes and is faced with an increasing number of pesticides and their degradables/ metabolites which combine with other physical natural factors and losses of biodiversity to intimidate almost all ecosystems. This complex system of interactions demand massive trails and efforts to extend incorporated information on the status and development of environmental quality (Markert *et al*., 2003). Biomarkers and bioindicators are an excellent tools in these field and could provide information which cannot be derived from conventional measurements alone. Special attention is driven to biomarkers, subcellular systems such as enzymes that can be used to find out effects of pesticides on the performance of organisms. Researcher place extra emphasis on the use of bioindicators of reverse outcomes in ecological safety, safety assessment, environmental exposure and potential health risks(Biomarkers Definitions Working Group, 2001). There is great pledge on use serum enzymes as indicators of adverse health outcomes in safety assessment (Duramad *et al*., 2007; Collings and Vaidya, 2008; Tarrant, 2010). Fipronil is a new

insecticides belongs to the phenylpyrazole group with several utility in control of many agricultural and veterinary pests. Because fipronil is used both commercially and in home applications, this leads to a high rate of potential contamination of the human domestic environment. Recent concerns for potential adverse public health effects of fipronil have been raised (Jennings *et al*., 2002;Anadón and Gupta, 2011; Mossa *et al*., 2015). Fipronil sulfone, a main metabolite of fipronil in both insects(Grant *et al*., 1998; Hainzl *et al*., 1998) and mammals, humans (Mohamed *et al*., 2004), mice (Hainzl and Casida, 1996) and rats (Cravedi *et al*., 2013), binds strongly to GABA receptors. On the other hand fipronil sulfone is also the degradation product in soil and water, formed throughabiotic processes; reduction, hydrolysis, photolysis and oxidation, respectively (Ngim and Crosby, 2001; Ying and Kookana, 2002). Previous studies have shown that fipronil sulfone exhibits greater persistence in environment (Hamon *et al*., 1996; Lin *et al*., 2008 and 2009; Konwick et al., 2006; Kuma and Singh, 2013) and has higher toxicity than the parent compounds(EPA, 1996; Hainzl *et al*., 1998; Schlenk *et al*., 2001; Tingle *et al*., 2003; Qu *et al*., 2016) to freshwater invertebrates, mammals, fish, birds and aquatic organism. For example, sulfone metabolite was found to have a 6- fold greater binding affinity than fipronil compound for GABA receptors in brain (Hainzl *et al*.,1998) and a 20-fold higher potency to block GABA-activated chloride channels in rats (Ikeda *et al*., 2001; Zhao *et al*., 2005). The fipronil-sulfone metabolite is 6.6 times more toxic to freshwater invertebrates, than fipronil itself (EPA, 1996).

Furthermore fipronil sulfone was shown to persist much longer in animal's tissues and blood than parent compound itself (Leghait *et al*., 2009 and 2010; Roques *et al*., 2012; Cravedi *et al*., 2013). Although the sulfone metabolite of fipronil exhibits greater persistence than fipronil, the available information on risk assessments of its metabolites remains limited that raises the question of the potential toxicity of this metabolite. Our previous results (Mossa *et al*., 2015) indicated that fipronil is a potent inhibitor of rat's biomarkers enzymes in serum. In contrast, no clear effect of fipronil metabolite was evidenced on the activities of biomarkers enzymes obtained from fipronil sulfone-treated rats.

The endocrine-disrupting effects of fipronil have been reported (Leghait *et al*., 2009).The decrease in thyroxine (T4) plasma concentrations was actually observed at a lower dose and shorter duration (FAO/WHO, 1997; Hurley, 1998). Low doses of fipronil produced greater effects when given for 14 days compared to a single dose (Moser *et al*., 2015). Changes in endocrine hormones and relative amounts of fipronil sulfone also varied between the dosing manners and need more investigation. Moreover, the combined toxicity of the parent compound and its toxic metabolites increase the risk of fipronil to organisms. Fipronil was introduced in Egypt market in the last few years and has been recommended to use for pest control in agriculture and domestic pests. Unfortunately, little information are available regarding the oxidative injury and thyroid hormones effect related to fipronil sulfone. Therefore, the present study was conducted to investigate the *in vivo* effects of fipronil sulfone on oxidative stress biomarker enzymes and

thyroid hormone-disrupting in male albino rats following repeated doses.

MATERIAS AND METHODS Chemicals

Fipronil-sulfone (99%purity), (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4 (trifluoromethylsulfonyl)-1-H-pyrazole-3-carbonitrile was obtained from Central Laboratory of pesticides, Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation of Egypt (MALR).

Kits used for the following biochemical assays, aspartate aminotransferases (AST, EC 2.6.1.1.), alanine aminotransferases (ALT, EC 2.6.1.2), alkaline phosphatase (ALP, EC 3.1.3.1), lactate dehydrogenase (LDH, EC 1.1.1.27), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPx, EC 1.11.1.9), glutathione-s-transferase (GST, EC 2.5.1.13), glutathione reduced (GSH), lipid peroxidation (LPO), albumin, uric acid, creatinine and total protein were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. All other chemicals were of reagent grades and were obtained from reputed companies.

Animals and experimental design

Twenty male albino rats, weighing 230 ±10 g were used in this study. The animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Dokki, Giza, Egypt. They were housed under normal environmental conditions of temperature and humidity and allowed to adapt to the new environment for two weeks before starting the experiment. Animal rooms (23±2 Cº) were maintained on a 12:12h light/dark photoperiod. Animals were provided with food with free access standard pellet diet and water *ad libitum*.

The animals were assigned randomly to 4 groups, 5 animals each, one control group and three fipronil sulfone treated groups. Experimental animals were treated with single daily oral administrations of fipronil sulfone at dose levels of 0.01, 0.1 and 1.0 mg/kg bw/day for 28 days, in corn oil. Fipronil sulfone was dissolved in 0.5 mL of corn oil and was daily administered orally by gavage (gastric intubation using syringe). The fipronil sulfone solutions were protected from light and were daily prepared before each administration. Control group was daily administered orally 0.5 mL of corn oil by gavage for 28 days.

Blood samples and tissue preparation

Rats were fasted overnight at the end of the experiment and blood samples were collected by puncturing the retero-orbital venous plexus of the animals with a fine sterilized glass capillary in clean dry tubes. Blood samples were left to clot and centrifuged at 3000 rpm (600 xg) for 10 min at 4°C using cooling centrifuge (Model universal 30 RF, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) to obtain the sera. Serum samples were stored at −20◦ C for further biochemical analysis, such as AST, ALT, ALP, LDH, albumin, uric acid, creatinine and total protein.

Rats were sacrificed by cervical dislocation and then liver was excised immediately and cleaned in saline solution. Liver samples were separately homogenized in 10% (w/v) ice cold 100 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm (2000 ×g) for 15 minutes at 4°C. The supernatant was obtained and kept frozen until used for oxidative stress biomarkers studies (SOD, CAT, GST, GPx, GSH, and LPO).

Biochemical measurements:

Liver function biomarkers; serum AST and ALT were determined according to the methods of Reitman and Frankel (1957), while ALP and LDH were measured according to Young *et al*. (1975) and Vassault (1983), respectively. Albumin, uric acid and creatinine were determined according to Westgard and Poquette (1972), Tietz *et al*. (1994) and Tietz (1995), respectively.

The determination of oxidative stress biomarkers were performed according to the kit's instructions. The principal of each determined biochemical parameter method is presented in Mossa *et al*., 2015. SOD was determined according to the method of Nishikimi *et al*. (1972) and activity was expressed as units/mg protein. CAT was determined according to the method of Aebi (1980) and activity was expressed as µmol/mg protein. GST was determined according to the manufacturer's instructions referred to Habig *et al.* (1974) and activity was expressed as µmol/mg protein. GPx was determined spectrophotometrically according to the method of Paglia and Valentine (1967) and activity was expressed in units/mg protein. GSH level was assessed spectrophotometrically according to the method of Beutler *et al.* (1963) and content was expressed in µmol/mg protein. Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content by a colorimetric method according to Satoh (1978). The MDA values were expressed as nanomoles of MDA/g protein. Protein concentration in homogenate was determined according to the method described by Lowry *et al.* (1951).

The triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (thyrotropin, TSH) in serum were measured by immunoassay analyzer using specific kits of "Human" (Germany). Triiodothyronine and thyroxine were expressed in ng/ml and thyroid-stimulating hormone was expressed as µIU/ml.

Statistical analysis

Data are presented as mean $\pm$ standard error (SE). Student's t-test was performed at $P< 0.05$ and $P < 0.01$ to determine the significant differences among the different treatments.

RESULTS AND DISCUSSION

Animal behavior and signs of toxicity

Non-mortality was observed during the experimental period in rats exposed to the fipronil sulfone at different concentrations.No abnormal reactions to handling and behavior were noted at the doses of 0.01, and 0.1 mg/kg bw/day. While, signs related to fipronil sulfone-treatment (1.0 mg /kg bw/day) included a change in activity and abnormal gait were observed from the first week. Moser *et al*. (2015) found that the high-dose (2 mg/kg bw/day) of fipronil sulfone showed weight decrease in the first four days, but this impact return to a normal state after the first week of dosing. The potential for fipronil sulfone to stimulate specific neurotoxicity was reported by other studies in rats (Hainzl *et al*., 1998; Zhao *et al*., 2005; Li and Akk, 2008). Zhao *et al*. (2005) documented that fipronil sulfone is more effective than fipronil to block GABAA receptor currents in rat neurons, dorsal root ganglion (DRG). Li and Akk, 2008, found that the kinetic potent effect of fipronil sulfone was almost identical to that of parent compound, but the metabolite was 10-time less potent in producing channel inhibition.

Body and relative organs weights

Data of final body weights, body weights gain and relative liver and kidney weights of male rats subjected to different treatments are shown in Table 1. Male rats exposed to fipronil sulfone showed non-significant differences in final and body gain weights compared to control treatment after 28 day of treatment (Table 1). In case of relative liver weights, all tested doses showed significant alterations in rats. Moreover, the relative kidney weights of rats exposed to 1.0 mg/kg bw/day showed highly significant alteration.

The results of this investigation clearly show that single daily orally exposure to fipronil sulfone partially reduced body weight and significantly altitude relative liver and kidney weights especially at highest dose (1.0 mg/kg). The increase in relative liver and kidney weights may be referred to the reduction in body weight gain of experimental animals or the relationship between the several toxicological effects and the liver weight increase (Banerjee *et al*., 1999; Mossa *et al*., 2015).

Biochemical enzyme in serum

As shown in Table (2), the biochemical parameters; total protein, albumin, uric acid and creatinine were determined in serum of male rat exposed to 0.01, 0.1 and1.0 mg/kg bw/day of fipronil sulfone for 28 days. The obtained results of the rat exposed to 1.0 mg/kg showed significant changes in the activity of total protein, uric acid and creatinine. Mostly, increase uric acid level takes place when kidneys don't eliminate uric acid efficiently. Things that may cause this slow-down in the removal of uric acid include inability of the kidneys to filter waste (Mossa and Abbassy, 2012), may be due to degradation of purines and pyrimidines, thyroid disruption (Leghait *et al*., 2009; Herin *et al*., 2011) and chronic exposure to fipronil (Tingle *et al*., 2003). In addition, the increase of total protein may be due to the abnormality or impairment in the function of liver and kidney with the high elevation of the serum enzyme (Mansour and Mossa, 2009 and 2010).

Creatinine is an excess product of creatine breakdown, which produced by muscles. The kidneys filter almost all of it from the blood and eliminate it into the urine. Results from a blood creatinine test are used to evaluate kidney function (Kassirer, 1971; Mossa *et al*., 2011; Mossa *et al*., 2015).

Others have also shown that, with repeated dosing, the sulfone metabolite (Leghait *et al*., 2009 and 2010; Lacroix *et al*., 2010; Roques *et al*., 2012) could persist much longer in the organism than fipronil itself and bioaccumulate in high lipid tissues (Cravedi *et al*., 2013).

It was found that the levels of protein and creatinine were significantly increased at all administrated doses, while albumin levels showed non-significant changes compared to control values. The level of uric acid were highly significantincreased, when rats were administrated 1.0 mg/kg of fipronil sulfone for 28 days.

Table 1. Body and organs weights of rats exposed to fipronil sulfone.

Treatments mg/kg	Body weight			Relative liver weight (%)	Relative kidney weight (%)
	Initial (g)	Final (g)	BW gains (%)		
Control	234.37±2.17	311.43±4.07	32.88±1.18	1.53±0.11	0.37±0.01
0.01	232.92±1.77	311.94±3.44	33.94±1.51	1.87±0.03*	0.39±0.01
0.1	230.24±1.65	313.34±5.11	36.14±2.63	2.09±0.04**	0.38±0.03
1.0	226.39±1.59	305.81±4.34	35.14±2.62	2.47±0.06**	0.56±0.04**

Each value is a mean of 5 animals ± S.E.; significantly different from control: *$p < 0.05$, **$p < 0.01$. Body weight gain (%) = [(Final B. Wt. - Initial B. Wt. / Initial B. Wt.)X100]; relative weight = (Organ weight/ Body weight) X 100.

Table2. Effect of 28-day fipronil sulfone treatments on albumin, total protein, uric acid and creatinine levels in serum of male rats.

Treatmentsmg/kg	Total protein(g/dl)	Albumin(g/dl)	Uric acid(mg/dl)	Creatinine(mg/dl)
Control	6.80 ±0.09	4.21 ±0.09	6.51±0.08	0.73 ±0.07
0.01	7.8 ±0.13**	4.09 ±0.06	6.50±0.14	0.76 ±0.01*
0.1	8.79 ±0.07**	4.17 ±0.12	6.53 ±0.08	0.80±0.03**
1.0	9.16 ±0.21**	4.26 ±0.24	7.71 ±0.13**	0.90±0.01**

Each value is a mean of 5 animals ± S.E.; significantly different from control: *p<0.05, **p < 0.01.

After 28 days of exposure to 0.01, 0.1 and 1.0 mg/kg of the fipronil sulfone, the activities of AST, ALT, ALP and LDH were highly significant increased compared to the control values Table (3). According to the data in Table (3) the increases in the enzymes activities were found to be dose-dependent manner.

The results of the present study indicate that singly daily repeated orally doses of 0.01, 0.1 and 1.0 mg/kg bw/day of fipronil sulfone for 28 days significantly increased serum biomarkers enzymes in rats. The aminotransferases enzymes, AST and ALT infiltrate into the blood if the hepatocytes are damaged. A chronic toxicity of the liver marked by degeneration of cells, inflammation, and fibrous thickening of tissue were observed in liver and kidney tissues of rats treated with fipronil (Mossa *et al*., 2015).

The AST is existing in a set of organs tissues in the body including brain, liver, kidney, heart and the muscle. It is leak into the serum in case of organ disruption. Therefore, it is not a specific biomarker of liver injury, while its elevation may be can exist as a result of other injured organs. On the other hand, ALT is broadly found in the liver tissues and released into the bloodstream as the result of liver injury (Awad *et al*., 1998). Thus, it serves as a fairly specific indicator of liver status. Because AST is located in many places in the body, high levels of AST alone don't suggest liver toxicity. However, the ratio of AST to the ratio of ALT, extends many proofs to what's going on inside.

According to the data in Table (3) rats exposed to 1.0 mg/kg bw/day of fipronil sulfone showed increased 54 and 152% for AST and ALT, respectively compared to the untreated rats. While, the rats exposed to 0.01 mg/kg bw/day showed increased by 9 and 20% for AST and ALT, respectively compared to the untreated rats. In the present study, treatment of albino rats with fipronil sulfone clearly

increased the AST and ALT release in a dose-dependent manner. Transaminases (AST and ALT) are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential functions (Seven *et al*., 2004).

In the present study, treatment of albino rats with fipronil sulfone increased the ALP and LDH release in a concentration-dependent manner. According to the available data and our previous data (Mossa *et al*., 2015) the expected toxicity of the fipronil sulfone should be higher than the fipronil itself. The results are supported by other studies conducted on fipronil and its metabolites (Chen *et al*., 2010; Romero *et al*., 2016). Liver enzymes in serum e.g., AST, ALT, ALP and LDH are mainly used in the evaluation of hepatic damage. In the current study, the elevation in LDH activity in serum of rat exposed to fipronil sulfone may be due to the hepatocellular necrosis and leakage of the enzyme into the blood (Goel *et al*., 2005; Mossa *et al*., 2015). The present study is parallel with the results of Ola *et al*. (2013) who found that LDH, AST and total plasma proteins were significantly increased in plasma of male buffalo calves at dose of 0.5 mg/ kg/ day fipronil sulfone for 21 days.

Table3. Effect of 28-day fipronil sulfone treatments on liver biomarkers enzyme in serum of male rats.

Treatmentsmg/kg	AST (U/L)	ALT(U/L)	ALP(U/L)	LDH(U/L)
Control	55.51±0.15	25.16±0.52	59.50±0.31	109.37±0.67
0.01	60.31±0.16**	30.33±0.17**	62.27±0.40**	112.89±1.05*
0.1	65.13±0.25**	45.80±0.42**	75.40±1.22**	149.61±1.33**
1.0	85.11±0.23**	63.24±0.67**	103.75±1.23**	200.88±2.17**

Each value is a mean of 5 animals ± S.E.; significantly different from control: *$p < 0.05$, **$p < 0.01$.

Oxidative stress biomarker

In the present study the oxidative stress biomarkers; SOD, CAT, GST, GPx, GSH and LPO were determined in liver tissues of male rats exposed to 0.01, 0.1 and 1.0 mg/kg b. wt. of fipronil sulfone for 28 days (Table 4). It was found that the fipronil sulfone treatments caused statistically significant changes in the activities of the oxidative stress biomarkers in the liver tissue dependent on the fipronil sulfone dose. No changes were observed in the activity of SOD, CAT and GSH at the treatments of 0.01 mg/kg of fipronil sulfone when compared to the untreated rats. While significant changes were observed in the activity of GST, GPx and LPO. Rat exposed to 0.1 mg/kg showed significant changes in the activity of GST, GPx, GSH and LPO and no significant changes in the activity of SOD and CAT. Highly significant changes in the all tested biomarkers were observed in liver tissues at the treatment of 1.0 mg/kg.

Cell metabolism produce reactive oxygen species (ROS) in normal case (McCord, 1993). Low levels are necessary for cell functioning, whereas immoderate generation of these products can adversely affect cell functioning (Gregorevic *et al*., 2001; Fattman *et al*.,

2003). Both production of lipid peroxidation (LPO) and ROS occurs in toxicological studies such as pesticides exposure. Fipronil can be responsible for increases in the production of ROS in cells, which lead to increased lipid peroxidation levels and oxidative stress (Bolton *et al.*, 2000). In human cell MDA is conceded one of the final products of LPO, which increase also by overproduction of ROS and representative as biomarker of oxidative stress (Draper and Hadley, 1990; Nielsen *et al*., 1997). For that reason, it has used as indicators of pesticides induced oxidative stresses (Kelly *et al.*, 1998).

In the current study, increased levels of MDA produced in the liver of fipronil sulfone-treated rats may be due to excess production of hydroxyl radicals, reactive oxygen metabolites and convert antioxidant defense system (Banerjee *et al*., 1999). In a harmony with our investigation raised lipid peroxidation levels were observed in *C. carpio* (Ki *et al*., 2012). SOD is the major antioxidant enzyme, particularly convert superoxide radicals to hydrogen peroxide (Andersen *et al*., 1997), together with GPx and CAT detoxify hydrogen peroxide to water (Inoue, 1994). Superoxide anion, hydrogen peroxide, and hydroxyl radical (reactive oxygen species) play an important role as agents in complicated processes when they are created exceedingly or when enzymatic and non-enzymatic defense systems are deteriorated (Michiels *et al*., 1994). The excessive levels of both SOD and CAT in the liver tissues of male rats at the high dose, 1.0 mg/kg were created excessively when the defense enzymes are deteriorated. However, fipronil sulfone did not cause significant changes in the SOD and CAT activities to rat exposed to 0.01 and 0.1 mg/kg.

GST conceded an important group of enzymes, which has an important role in catalysts conjugation compounds with glutathione (Chasseaud, 1979; Motoyama and Dauterman, 1980). GSTs act as biomarkers in many investigations of several invertebrates and vertebrates (Castro *et al*., 2004; Monteiro *et al*., 2006). On the other hand, GST is not a sensitive biomarker of fipronil exposure or effect in zebrafish (Wu *et al*., 2014). In the current study the GST activities significantly differed between the tested concentrations and the control in the tested tissues. The dose responses in GST suggested that GST is a sensitive biomarker of fipronil sulfone exposure or effect in in rats.

In this study, various concentrations of fipronil sulfone induced oxidative stress enzymes activity in dose-dependent manner. These results suggested that SOD, CAT, GST, GPx, GSH and LPO could be used as a biomarker of fipronil sulfone effects. Unfortunately, limited information is available on risk assessments, pharmacokinetics and tissue distribution of fipronil and its metabolite fipronil sulfone (Lu *et al*., 2015; Romero *et al*., 2016).

Thyroid function hormones

Table (5) shows the mean (±SE) total of triiodothyronine (T3), total thyroxine (T4) and thyroid-stimulating hormone (TSH) serum in control and fipronil sulfone-treated groups after 28 days of treatment. The obtained results showed high significant decreases in the levels of total T3 and T4 concentration in male rats serum exposed to the all fipronil

sulfone treatments. T3 and T4 serum concentrations were 49 and 43% lower in fipronil sulfone treated rats, respectively at 1.0 mg/kg as compared to untreated rats after 28 days of treatment. However, fipronil sulfone treatments were associated with high significant increases in serum TSH levels at all the tested doses. Prevouis studies reported decreased thyroid hormones with repeated dosing (Leghait *et al*., 2009; Roques *et al*., 2012). Others have also shown that, with repeated dosing, the sulfone metabolite persistence higher in organisms (APVMA, 2009; Leghait *et al*., 2009; Lacroix *et al*., 2010) .The increased levels of the sulfone metabolite may be related to induction of hepatic metabolizing enzymes (Das *et al*., 2006). Other study shown that, in the thyroid hormone receptor (TR) assay, fipronil sulfone showed anti-thyroid hormone activity with the relative luciferase activity of 8.2×10^{-7} M (Lu *et al*., 2015). The low observed effect level (LOEL) value of fipronil sulfone from the TR assay as 45.3 ppb (1.0×10^{-7} M = 45.3 ppb). The previous studies indicated that the concentration of fipronil sulfone detected in sediments was as high as 11 ppb (Brennan *et al.*, 2009), it seems that the residual value of fipronil sulfone in environment can make an adverse effect on organisms, but more relevant researches are required for the comprehensive risk assessment of fipronil sulfone. Considered Maximum residue levels (MRLs) in food products range from 0.01 to 1.5 ppm depending on the animal or crop product. The presence of fipronil containing products in many households suggests that the possibility of contamination and efficient ways to treat it should be considered (Jennings *et al*., 2002).Accidental human exposure to fipronil has indicated that the ratio of fipronil sulfone/fipronil plasma concentrations at 24 h post-ingestion is about 0.25–0.5 (Mohamed *et al*., 2004).

Table 4.Effect of 28-day fipronil sulfone treatments on oxidative stress biomarker of male rat.

Treatments mg/kg	SOD (U/mg protein)	CAT (µmol/ mg protein)	GST (µmol/ mg protein)	GPx (U/ mg protein)	GSH (µmol/ mg protein)	LPO(nmol/m g protein)
Control	6.49±0.15	13.28±0.17	0.46±0.014	7.02 ±0.25	0.072±0.003	73.1 5±2.76
0.01	6.76±0.15	13.79±0.36	0.40±0.016*	5.60 ±0.16**	0.067±0.002	78.85±1.44**
0.1	6.82±0.14	12.58±0.18	0.40±0.006**	5.28±0.19**	0.061±0.002*	90.54±3.78**
1.0	4.58±0.15**	7.27±0.37**	0.34±0.033**	3.48 ±0.23**	0.043±0.002**	111.76±4.98**

Each value is a mean of 5 animals ± S.E.; significantly different from control: *$p < 0.05$, **$p <$; superoxide dismutase; CAT: catalase; GST: glutathione-S-transferase; GPx: glutathione peroxidase; GSH: glutathione reduced; LPO: lipid peroxidation.

Table 5.Effect of 28-day fipronil sulfone treatments on T3, T4 and TSH hormones in serum of male rats

Treatments mg/kg	T3 (ng/ml)	T4 (ng/ml)	TS (µIU/ml)
Control	0.63±0.03	55.17±1.22	0.05±0.01
0.01	0.47±0.01 **	44.64±1.29 **	0.09±0.01 **
0.1	0.36±0.01 **	35.95±0.77 **	0.15±0.02**
1.0	0.32±0.01 **	31.35±0.90 **	1.66±0.09 **

Each value is a mean of 5 animals ± S.E.; significantly different from control: *$p < 0.05$, **$p < 0.01$; T3:triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone.

CONCLUSION

From the current study, it has been concluded that the exposure to fipronil sulfone significantly impact on oxidative stress biomarkers in rat and thyroid hormones in male albino rats. Further investigations are required to determine the contribution of fipronil sulfone to mammalian toxicological effects.

Conflicts of interest

There are no conflicts of interest to declare.

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تأثير الفبرونيل سلفون على مؤشرات الاكسدة الحيوية وهرمونات الغدة الدرقية في فئران التجارب

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تم تقييم التأثير السام لمركب الفبرونيل سلفون على الفئران البيضاء لبعض الأنزيمات, البروتينات بالدم ومؤشرات الاكسدة الحيويه مثل superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) glutathione peroxidase (GPx), glutathione reduced (GSH) and lipid peroxidation (LPO) فى انسجة الكبد بالإضافة الى تأثيره على هرمونات الغدة الدرقية حيث قسمت الفئران الى اربعة مجاميع كل مجموعة تضمنت خمسة فئران. حيث تم حقن الفئران بجرعات الفبرونيل سلفون 0.01, 0.1 و 1.0 ملليجم/كجم من وزن الجسم يوميا من خلال التجويف الفمى لمدة 28 يوم متتالى. وقد اوضحت النتائج وجود تأثير معنوى على مستويات البروتين والكرياتنين تبعا للجرعات المستخدمه، وكذلك تأثرت بمعنوية عالية انزيمات وظائف الكبد مثل aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). اما بالنسبة لتأثير المركب على مؤشرات الاكسدة الحيوية مثل SOD, CAT, GST, GPx, GSH و LPO فى انسجة الكبد

فقد اوضحت النتائج ان الجرعة العالية 1 ملليجم/كجم من وزن الجسم يوميا من خلال التجويف الفمى لمده 28 يوم متتالية من المركب ادت الى تغيرات عاليو المعنوية. وعند تقدير هرمونات الغده الدرقيه وجد ان جميع الجرعات تحت الدراسه اظهرت تأثر مستويات هرمون thyroid-stimulating hormone (TSH) فى الدم بالزياده بينما اظهر كل من هرمون triiodothyronine (T3) and thyroxine (T4) نقصا معنويا بالمقارنة بالفئران الغير معاملة.

توصى الدراسه الحالية بالحاجة إلى المزيد من اجراء البحوث لإستيضاح مساهمة الفبرونيل سولفون ناتج التمثيل الحيوى الناجم من استخدام مبيد الفبرونيل على اضطراب الغدة الدرقية وتأثيرهم على المؤشرات الحيوية وارتباطهم بالمسارات البيولوجية فى فئران التجارب .