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Research Article
Ameliorating Effect of Zinc Against Oxidative Stress and Lipid Peroxidation Induced by Fipronil in Male Rats

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Abstract

Objective: The present study was carried out to investigate the protective effect of zinc against oxidative damage induced by fipronil (FPN) in the liver and kidney of male rats. **Methodology:** Rats were assigned to four groups and received, water (control group), fipronil at concentration 10 mg L^{-1} (2.0 mg kg^{-1} b.wt.), zinc at concentration 227 mg L^{-1} and fipronil plus zinc group. All treatments were administered through drinking water for 45 days. The concentration used of fipronil in this study represents 2.0 mg kg^{-1} b.wt., based on average water consumptions and body weights of treated rats. **Results:** Results revealed that fipronil induced insignificant changes in body weight and significant increase in liver weight of treated rats. Also, significant activities of oxidative stress enzymes such as, superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GPx), catalase (CAT) and level of glutathione reduced (GSH) were obtained. The FPN caused a significant elevation in lipid peroxidation level. However, the adverse effects induced by FPN were mitigated when the rats were supplemented with zinc. Moreover, three new protein bands were detected only in the electrophoretic pattern of the FPN treated group and disappeared in the remaining three groups; control, zinc and fipronil plus zinc. **Conclusion:** The overall outcome suggested that FPN induced adverse effects on oxidant/antioxidant status in the liver and kidney of intoxicated rats and zinc has a role in attenuating these effects. These outcome show that administration of Zn may be useful, easy and economical to protect human against phenyl pyrazole insecticide toxic effects.

Key words: Fipronil, zinc, oxidative stress biomarkers, one-dimensional gel electrophoresis, albino rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fipronil (FPN) is a class of phenyl pyrazole pesticides used for the control of a wide range of agricultural, public health and veterinary pests, including many lepidopteran species as well as thrips, locusts, ants, cockroaches, fleas and ticks[1]. Because FPN is widely used in agriculture 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 FPN have been raised[2].

Pesticides can alter oxidant/antioxidant status and induce oxidative damage leading to the production of free radicals and changes in enzymatic and non-enzymatic antioxidants or the oxygen free-radical scavenging enzyme system[3-5]. Assayed *et al.*[6] studied that are describing the oxidative stress mechanisms in fipronil-induced toxicity are limited. Mossa *et al.*[7] pointed out that FPN caused histopathological alterations in liver and kidney of male rats via induction of lipid peroxidation, oxidative stress, liver and kidney injury. They attributed pathphysiological changes to generation of free radicals, Reactive Oxygen Species (ROS).

The FPN at dose 10, 5 and 2.5 mg kg^{-1} b.wt., induced significant increase in the level of lipid peroxidation (LPO) and decrease in the activity of antioxidant enzymes such as SOD, CAT and GST. Also, the content of non-enzymatic antioxidants such as GSH and total thiol were decreased[5]. Currently, study of oxidative damage via estimated of free radical generation and antioxidant defense has become an important aspect of investigation in mammals. Also, some studies investigated the potential protective effects of some natural antioxidant, such as vitamin C[8], isoflavones[9] and zinc[3] against oxidative damage induced by pesticides in mammals. In this respect, researchers have shown that zinc, as an essential nutrient, under certain conditions may have antioxidant properties[10]. Zinc plays a crucial role in regulation of cellular glutathione that is essential for antioxidant defense in human and animals[11].

In our previous study[7], we studied the relationships between exposure to FPN and oxidative stress biomarkers in the liver and kidney of male rats. It had a significant alteration in oxidative damage parameters and the high effect was recorded in rats treated with 2 mg kg^{-1} b.wt. Moreover, chronic exposure to FPN induced thyroid, liver and kidney dysfunction and damage in rat[1]. However, there are no available studies on the opposite effects of zinc on fipronil toxicity.

To the best of our knowledge, this is the first study to evaluate the protective role of zinc against oxidative damage induced by FPN in the liver and kidney of male rats. Therefore, the objective of the present study was designed to investigate the ameliorating effect of zinc on nutralizing the oxidative stress and lipid peroxidation induced by FPN in the liver and kidney of male rats together with changes occurred in the serum protein profile.

MATERIALS AND METHODS

Chemicals used: Fipronil (Insecto SC 5%) is a product of BASF Company and manufactured by Sinochem Group-Ningbo Technical Co. Ltd., China. Zinc was obtained (in the form of zinc sulphate heptahydrate) from October Pharma, Egypt. The kits used for the following biochemical assays were purchased from Biodiagnostic Company, Dokki, Giza, Egypt: 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) and total protein. All other chemicals were obtained from reputed companies.

Animals and experimental design: Twenty male albino rats, weighing 90±10 g (6 weeks age) 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 2 weeks before starting the experiment. Animal rooms (23±2°C) were maintained on a 12:12 h light/dark photoperiod. Animals were provided with food with free access standard pellet diet and water *ad libitum*. All animal procedures were conducted according to accepted standards of animal care following NODCAR Guidelines. Rats were randomly divided into four groups, five rats in each group. Control group, rats received drinking water. Zinc group, rats received zinc at concentration 227 mg L^{-1} in drinking water. Fipronil group, rats received FPN at concentration 10 mg L^{-1} in drinking water. The FPN plus zinc group, rats received drinking water contain FPN and zinc at concentration 10 mg L^{-1} of FPN and 227 mg L^{-1} of zinc for 45 days. The selected dose of FPN was based on our previous study in which 10 mg L^{-1} fipronil in drinking water for 45 days induced biochemical and histopathological alterations in liver and kidney of male rats[11], while that of zinc referred to Goel *et al.*[12] and Mansour and Mossa[3]. Concentration of FPN and zinc were daily prepared and body weights were monitored weekly during the experimental period. The concentration used of FPN in this study represent 2.0 mg kg^{-1} b.wt., based on average water consumptions and body weights of treated rats.

Blood samples and tissue preparation: The rats were fasted overnight at the end of the experimental period 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. After blood collection, the rats were sacrificed by cervical dislocation. Blood samples were left to clot and centrifuged at 3000 rpm (600×g) for 10 min at 4°C using Hettich universal 30 RF (Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany) to obtain the sera. Serum samples were stored at -20°C for protein analysis by gel electrophoresis. Liver and kidney were excised immediately and cleaned in saline solution. Then the liver and kidney 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 min at 4°C. The supernatant was obtained and kept frozen until used for oxidative stress biomarkers studies (SOD, CAT, GST, GPx, GSH and LPO) and total protein.

Biochemical measurements: Determination of oxidative stress biomarkers includes SOD, CAT, GPx, GST, GSH and LPO was performed according to the kit's instructions. The principal of each method isgiven below for each determined biochemical parameter. The SOD was determined according to the method of Nishikimi *et al.*[13]. The method based on the ability of SOD enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Briefly, 0.05 mL^{-1} sample was mixed with 1.0 mL^{-1} buffer (pH 8.5), 0.1 mL^{-1} nitroblue tetrazolium (NBT) and 0.1 mL^{-1} NADH. The reaction was initiated by adding 0.01 mL^{-1} phenazine methosulphate (PMs) and then absorbance was read at 560 nm for 5 min. The SOD activity was expressed as U mg^{-1} protein. The CAT was determined according to the method of Aebi[14]. The method is based on the decomposition of H_2O_2 by catalase. The CAT activity was expressed as µmol mg^{-1} protein. The GST was determined according to the manufacturer's instructions referred to Habig *et al.*[15]. The method was based on the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH) in a reaction catalyzed by GST. The GST activity was expressed as µmol mg^{-1} protein. GPx was determined spectrophotometrically according to the method of Paglia and Valentine[16]. The estimation of GPx activity was based on the oxidation of GSH and NADPH using glutathione reductase (GR) and measuring the decrease in absorbance at 340 nm and expressed in U mg^{-1} protein. The GSH level was assessed spectrophotometrically according to the method of Beutler *et al.*[17]. The method was based on the reduction of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound. The GSH content was expressed in µmol mg^{-1} protein. Lipid peroxidation was estimated by measuring Thio Barbituric Acid Reactive Substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content by a colorimetric method according to Satoh[18]. The MDA values were expressed as nanomoles of MDA per gram protein. Protein concentration in homogenate was determined according to the method described by Lowry *et al.*[19].

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE): Polyacrylamide gel electrophoresis of serum proteins was carried out according to Laemmli[20]. The slap SDS-polyacrylamide gel (10%) was used to separate the proteins extracted from rat serum. An equal amount of a protein (40 µg) sample was mixed at a ratio of 1:1 (v/v) with a sample buffer containing 0.1% bromophenol blue as a marker loaded in each well of the gel. The gel was run at a constant 100 V for 1 h then at a constant 200 V for 3 h. After the end of the run, proteins on the gel were fixed and stained overnight using commassie blue R 250. After the fencing of the destaining step until getting a clear background, the gel was immersed in the conserving solution for about 15 min and left for drying in solifan papers at room temperature. An image of the dried gel was done using an EPSON scanner perfection V550 photo, then transferred to an image analyzer, TotalLab Quant, to integrate the gel data.

Statistical analysis: Data in the present study were expressed as Mean±Standard Error (SE) and were statistically analyzed by one-way ANOVA analysis followed by Duncan's test using SPSS version 18.0 for windows and the differences were statistically significant at $p<0.05$.

RESULTS

No mortality occurred during the experimental period. The weekly body weight gain was insignificantly changed among treatments (Fig. 1a). In contrast, FPN treatment induced significant increase in relative liver and kidney weights in rats (Fig. 1b). However, co-administration of zinc in conjunction with FPN reversed the increase in relative liver and kidney weights of male rats but the increases were stillstatistically different the control.

Table 1 shows that oxidative stress biomarkers such as SOD, CAT, GST, GPx, GSH and LPO were determined in the liver tissues of male rats. Treatment of rats with zinc (zinc group) had no detectable significant effect on the activity of all determined biochemical parameters as compared to

control group. Rats exposed to FPN (FPN group) showed highly significant reduction in the activity of SOD, CAT, GST and GPx in liver tissue while level of GSH and LPO increased. The LPO level increased by 1.5 fold compared to untreated rats. Zinc in combination with FPN alleviated its harmful effect on the activities of the above measured biomarkers and maintained the activities or level close to near the normal values in the control group. There were slight significant differences among all determined enzymes as related to untreated group.

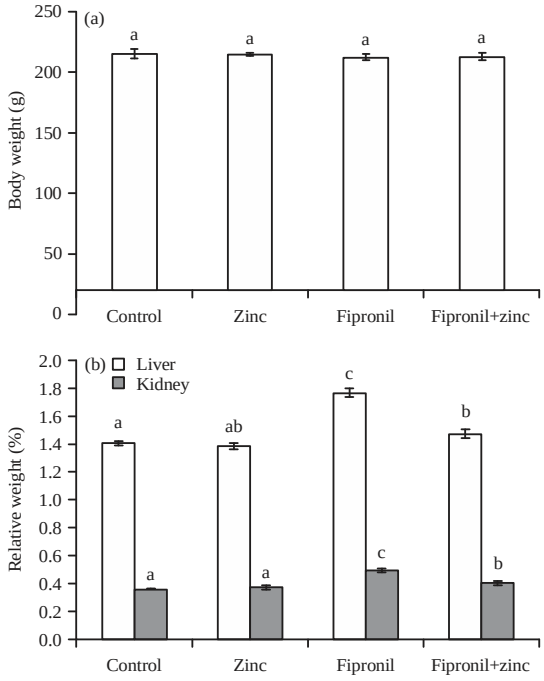

Fig. 1(a-b): (a) Body and (b) Relative liver and kidney weights of male rats exposed to fipronil and the protective role of zinc. Values are Means±SE, n: 5, values having the same letters is in significantly different from each others. Relative weight (%): [organ weight/body weight]×100

Similar results to the activity of the biochemical parameters determined in tissues were observed (Table 2). All oxidative stress biomarkers significantly decreased as compared to control group in FPN-treatment group except that for LPO level. With regards to zinc treatment, no significant effect on the activity of aforementioned stress parameters was observed when compared to the control group. When zinc was co-administrated to the FPN-treated groups, significant improvement in the activity of antioxidant enzymes (SOD, CAT, GST and GPx) and level of GSH and LPO was recorded . It remarkably normalized only the level of GSH but not for other determined biomarkers. The FPN had a similar effect on LPO level, which increased to be 1.6 fold more than that in the control.

Protein analysis and profiling by polyacrylamide gel electrophoresis (PAGE) of serum protein of untreated control and treated rat groups is illustrated in Fig. 2. Also, Table 3 shows the rate flow (R_f) values, molecular weight, calibration volume and the percentage of the protein band content in serum of rats treated with FPN, FPN-zinc, zinc and the control. The percentage content of the protein band with R_f values 0.077, 0.153, 0.197, 0.321, 0.839 and 0.869 decreased in the FPN-treatment. While a significant decrease was observed in the band with R_f value 0.439 as the calibration volume of this band was 36.71 μg. On the other hand, the

Table 1: Oxidative stress biomarkers in liver tissues of male rats exposed to fipronil and the protective role of zinc

	Oxidative stress biomarker					
Treatments	SOD (U mg^{-1} protein)	CAT (μmol mg^{-1} protein)	GST (μmol mg^{-1} protein)	Gpx (U mg^{-1} protein)	GSH (μmol mg^{-1} protein)	LPO (nmol mg^{-1} protein)
Control	6.87±0.03^{d}	13.86±0.12^{c}	0.44±0.01^{c}	7.68±0.19^{c}	0.077±0.001^{c}	73.81±0.59^{a}
Zinc	6.77±0.01^{c}	13.87±0.03^{c}	0.44±0.01^{c}	7.69±0.21^{c}	0.076±0.002^{c}	73.84±0.37^{a}
Fipronil	5.39±0.08^{a}	07.82±0.16^{a}	0.33±0.004^{a}	3.56±0.26^{c}	0.048±0.002^{a}	111.67±0.49^{b}
Fipronil+zinc	6.12±0.03^{b}	12.69±0.20^{b}	0.42±0.004^{b}	6.20±0.02^{b}	0.066±0.003^{b}	96.14±3.55^{c}

Each value is a mean of 5 animals ±SE, values have the same letter are insignificantly from each others, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-s-transferase, GPx: Glutathione peroxidase, GSH: Glutathione reduced, LPO: Lipid peroxidation

Table 2: Oxidative stress biomarkers in kidney tissues of male rats exposed to fipronil and the protective role of zinc

	Oxidative stress biomarker					
Treatments	SOD (U mg^{-1} protein)	CAT (μmol mg^{-1} protein)	GST (μmol mg^{-1} protein)	Gpx (U mg^{-1} protein)	GSH (μmol mg^{-1} protein)	LPO (nmol mg^{-1} protein)
Control	5.07±0.12^{c}	9.16±0.16^{d}	0.079±0.003^{c}	4.56±0.19^{c}	0.045±0.002^{b}	66.07±3.01^{a}
Zinc	4.85±0.08^{b}	8.79±0.23^{c}	0.076±0.004^{bc}	4.48±0.27^{c}	0.045±0.003^{b}	66.15±3.12^{a}
Fipronil	4.17±0.04^{a}	4.87±0.15^{a}	0.054±0.004^{a}	2.60±0.09^{a}	0.025±0.002^{a}	106.93±2.92^{b}
Fipronil+zinc	4.94±0.04^{b}	7.44±0.45^{b}	0.073±0.003^{b}	3.57±0.14^{b}	0.045±0.003^{b}	84.37±4.24^{c}

Each value is a mean of 5 animals ±SE, values have the same letter are insignificantly from each others, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-s-transferase, GPx: Glutathione peroxidase, GSH: Glutathione reduced, LPO: Lipid peroxidation

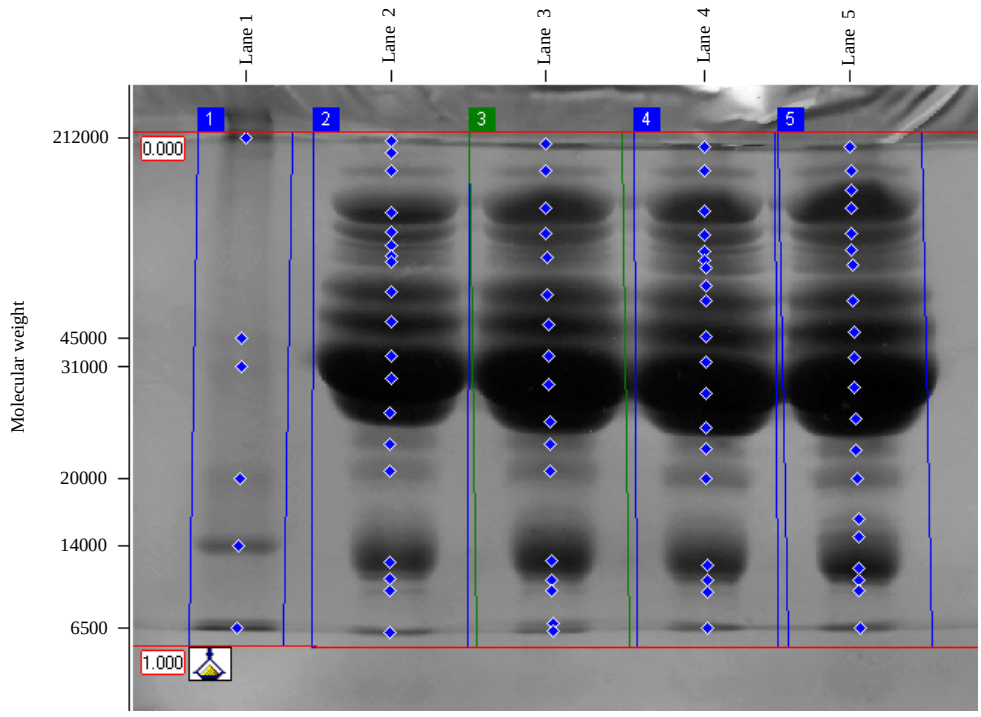

Fig. 2: Polyacrylamide gel electrophoretic (PAGE) profiles of protein marker and serum of treated groups. Line 1: Protein marker, line 2: Zinc, line 3: Control, line 4: Fipronil+zinc and line 5: Fipronil

Table 3: Relative content of each protein band fractionated from serum of treated rats compared to the control

		Control		Zinc		Fipronil		Fipronil+zinc	
R_f value	Molecular weight (MW)	CV (µg)	Band (%)	CV (µg)	Band (%)	CV (µg)	Band (%)	CV (µg)	Band (%)
0.025	178.320	100	1.92	117.14	2.15	105.26	2.1	106.99	2.34
0.039	169.207			N	1.52				
0.077	148.872	100	2.81	94.14	2.54	67.05	1.96	86.4	2.77
0.112	131.454					N	2.8		
0.153	114.213	100	10.11	107.43	10.41	79.97	8.41	85.38	9.83
0.197	97.800	100	4.96	93.73	4.46	81.66	4.22	86.24	4.87
0.227	87.990			N	2.12	N	1.97	N	1.45
0.245	82.853	100	3.46	46.96	1.56	ND	ND	21.45	0.85
0.259	78.780			N	2.75	N	3.53	N	3.41
0.229	68.680							N	2.18
0.321	63.575	100	9.81	89.13	8.38	81.36	8.31	61.36	6.86
0.382	51.408	100	8.99	109.32	9.42	99.55	9.32	79.44	8.13
0.439	42.136	100	9.32	91.25	8.15	36.71	3.56	42.82	4.54
0.493	34.948	100	17.74	80.52	13.69	100.73	18.6	109.87	22.2
0.561	27.645	100	4.48	159.49	6.85	125.12	5.84	74.4	3.8
0.612	23.113	100	2.77	151.4	4.02	130.69	3.77	95.33	3.01
0.666	19.164	100	4.42	109.25	4.63	102.09	4.69	110.27	5.55
0.752	14.150					N	2.18		
0.785	12.608					N	3.89		
0.839	10.492	100	11.3	97.54	10.56	70.87	8.33	88.89	11.44
0.869	9.431	100	2.81	114.32	3.08	69.76	2.04	85.51	2.73
0.891	8.743	100	1.35	137.57	1.78	142.28	2	93.57	1.44
0.959	6.895	100	1.85	ND	ND	ND	ND	ND	ND
0.970	6.659	100	1.91	105.47	1.93	128.31	2.48	123.81	2.61

R_f: Rate flow, ND: Band not detected, N: New band

electrophoretic pattern of the serum proteins showed an increase in the protein content of the band with a molecular weight (MW) 8.743 kDa (R_f 0.891), which increased by 142.28 µg with band 2% after the treatment with FPN

compared to the same protein band in the control group. Moreover, the band with MW 6.659 kDa, also shows an increase in protein content inbothFPN and FPN-zinc groups when compared to the same protein bands in the control group. Figure 1 also shows that new bands of certain proteins were detected in the electrophoretic pattern of FPN-treated group and disappeared in the control, zinc and FPN-zinc treated rats. These three new bands having R_f values 0.112, 0.752 and 0.785 and approximate molecular weights 131.454, 14.150 and 12.608 kDa, respectively (Table 3). The percentages of the protein band content in these new proteins were 2.8, 2.18 and 3.89%, respectively. In contrast, the new protein band was detected only in the FPN-zinc treatment with MW 68.680 KDa (R_f 0.229). The content of this band was 2.18%. In zinc treated group, one new band was also detected with approximate molecular weights 169.207 kDa and R_f value 0.039. Thus, this band may be present as a result of the zinc treatment (Table 3). On the other hand, the protein bands having R_f value 0.227 and 0.259 with approximate molecular weights 87.990 and 78.780 kDa, appeared only in the zinc, FPN-zinc and FPN-treatments. The FPN and FPN-zinc treatments elevated the protein content of the band having R_f value 0.259 by 3.41 and 3.53%, respectively. These proteins may have a role as a defense against oxidative damage caused by FPN-treatment.

The protein band with R_f value 0.959 appeared only in the electrophoretic pattern of the sera protein of the untreated rats. While, the protein band having R_f 0.245 was not detected only in the FPN-treatment. This protein band was lower in the FPN-zinc-treatment than that in the zinc-treatment by approximately 50%, the calibration volume was 21.45 and 46.96 µg, respectively. These proteins may be either newly synthesized or in a concentration below the limit of detection. According to data in Table 3, the treatment of FPN-zinc increased the content of some protein bandswith MW 148.872, 114.213, 97.800, 42.136, 10.492 and 9.431 kDa.

DISCUSSION

In our previous study[11], FPN induced significant alteration in oxidative stress biomarkers and lipid peroxidation in liver and kidney of male rats. Also, Tingle *et al.*[1] found that FPN induced rat organ toxicity such as, thyroid, liver and kidney after chronic exposure. The results of the present study indicated that exposure to FPN caused significant alteration in oxidative stress biomarkers in the liver and kidney of male rats, which was evident from the generation of free radicals. Also, a free radical is known to cause damage in cell membrane and component e.g., DNA and protein. It also implicated in the pathogenesis of various liver and kidney injuries[21,22]. Therefore enzymatic and non-enzymatic biomarkers were extensively used as a marker of oxidative stress[4,12,23-25]. So, oxidative stress is considered as a biomarker of pesticides induced oxidative stresses and suggested as one of the molecular mechanisms involved in pesticides-induced toxicity[26]. In the present study, increase level of LPO in liver and kidney of FPN-treated rats may be due to increased production of reactive oxygen species, mainly hydroxyl radicals that damage antioxidant defense system[27]. This data are consistent with those by Tukhtaev *et al.*[28] who found that prolonged exposure to low doses of FPN increased LPO in liver of pregnant rats and their offspring. The obtained results are also in line with with those by Mossa *et al.*[11] who pointed out that LPO was significantly increased in liver and kidney of FPN-treated male rats. They also proposed that increased LPO in the liver and kidney could be due to the toxic effect of FPN which is associated with the production of free radicals. Moreover, the decrease in the activity of SOD in FPN-treated rats may be attributed to the utilization of this enzyme (as an antioxidant) for converting the free radical formed $O^{\cdot}_2$ to H_2O[29,30]. The GST is an enzyme family responsible for metabolism of pesticides via catalyzing the conjugation of a variety of electrophilic substrates to the thiol group of GSH to producing less toxic forms[31]. The activity of GST was significantly diminished by almost 25 and 32 % in the liver and kidney, respectively. This lower activity may be because of the reduced metabolic activity of FPN and the damage caused by reactive oxygen species[12]. The FPN also decreased the activity of GST in tadpoles of frogs[32]. Similar results were observed in the liver of chlorpyrifos[3], diazinon[33] treated rats and in the plasma of deltamethrin treated rats[34].

The SOD, CAT and GPx are considered as the first defense system that protects cell components from oxidative damage caused by reactive oxygen species[21]. Thus, the decrease in SOD, CAT and GPx activity in the liver and kidney in rats exposed to FPN could be explained as excess formation of $O^{\cdot}_2$ which rapidly converted to H_2O by SOD and to water by CAT and GPx. The remarkable decline in SOD activity was also observed in liver of pregnant rats and their offspring exposed to low doses of FPN[28]. Previous studies also reported that exposure to insecticides such as chlorpyrifos[3], triazophos[21] and deltamethrin[9] resulted in a decrease in SOD, CAT and GPx in liver and kidney of rats.

Currently, several studies have shown that minerals such as zinc are very important for nutrition. Moreover, zinc is considered as an essential nutrient, at least under certain conditions. Zinc has also a role as co-enzyme of SOD and it

may have antioxidant properties[10]. Therefore, zinc can protect the cell membrane, DNA and protein against the damage induced by free radicals[35]. In the present study, results indicated that the supplement with zinc mitigated FPN-induced oxidative damage and decrease associated biomarkers changes in the liver of male rats. The activities of GST, SOD, CAT and GPx and the level of GSH were reduced in FPN-treated rats. Co-administration of zinc to FPN-treated rats improved oxidative stress biomarkers in the liver and kidney as compared to FPN-treated rats.

The protective effect of zinc on CAT activity in FPN-treated rats may be owed to antiperoxidative property of this metal ion, which in turn leads to decrease in production of superoxide radicals. The observed normalization trend in activity of GST after zinc treatment is likely because of either induced metallothionein content, or due to indirect action of zinc in reducing the levels of reactive oxygen species[36]. Zinc is known to induce the production of metallothionein, which is very rich in cysteine and is an excellent scavenger[37] of $OH^{\cdot}$. Presence of zinc with FPN alleviated its harmful effect on SOD activity that might be referred to the dismutation of $O^{\cdot}_2$ to H_2O which is catalyzed by SOD as it contains both copper and zinc. In addition, zinc is an inhibitor of NADPH oxidases which catalyze the production of $O^{\cdot}_2$ from oxygen by using NADPH as the electron donor[38]. The moderate normalization of LPO in the liver (86.5%) and in the kidney (80%) following zinc treatment is likely due to zinc antiperoxidative properties[39]. Moreover, a mixture of zinc and gingeravoid the toxic effect of malathion onthe liver and kidney of treated rats[40].

In our study, protein was separated by SDS-PAGE to determine the relative abundance of the major proteins in the serum of male rats and also to determine the distribution of proteins among different treatments (FPN, FPN-zinc and zinc). Thereby, the absence/presence of proteins in serum could be detected in each treatment. However, little information is available on the relationship between the toxic effect of FPN and its effects on protein. To the best of our knowledge, this is the first report to study the correlation between FPN-toxicity, impairment of the antioxidant system and protective effect of zinc, including changes of serum protein levels in rats. As shown in Table 3 and Fig. 2, three new bands of protein were detected in the electrophoretic pattern of FPN-group and not found in the control, zinc and FPN-zinc groups. The R_f values of these proteins were 0.112, 0.752 and 0.785 and approximate molecular weights were 131.454, 14.150 and 12.608 kDa, respectively. The formation of new protein may be due to the induction of microsomal enzymes, which metabolize xenobiotics as a correlation between the enzyme induction due to insecticidal treatment and protein biosynthesis was suggested[41]. The percentage content of the bands with R_f values 0.077, 0.153, 0.197, 0.321, 0.839 and 0.869 was decreased in the FPN-treatment. While a significant decrease was observed in the band with R_f value 0.439 and this may be related to the inhibition of some proteins. Saleem *et al.*[42] mentioned that decrease of total protein content may be related to the utilization of carbohydrates for energy production when insecticide exposure was continued. In the present study, rats exposed to FPN led to a significant reduction in the content of some protein bands. However, the relative protein volume increased in the rats exposed to FPN-zinc group. Consequently, zinc in combination with FPN alleviated the detrimental effect of FPN on the calibration volume of some proteins.

The band with MW 6.659 kDa showed an increase in protein content in both FPN and FPN-zinc-treated groups when compared to the control group. We suppose that this protein band may be related to the metallothionein protein. Metallothionein is a low molecular weight protein, isolated for the first time from horse kidney[43]. The mammalian forms are characterized by a molecular weight of 6-7 kDa, containing 60-68 amino acid residues. Metallothioneins were induced in many organs through the exposure to different factors such as cadmium, zinc, copper and mercury[44-46]. Also, metallothioneins were induced through the exposure to the herbicide paraquat[47] andinsecticide midacloprid[48]. Also, metallothioneins function as storage for different elements, which is important to activate many enzymes and hormone[49]. Beggel *et al.*[50] demonstrated a highly significant increase in metallothionein gene transcription over time and during the recovery period after 24 h of exposure larval fathead minnow (*Pimephales promelas*) to FPN concentrations of $\geq$31 μg L^{-1}.

CONCLUSION

Fipronil exposure produced a remarkable increase in LPO and decrease in enzymatic (SOD, CAT, GST and GPx) and non enzymatic antioxidant (GSH) in the liver and kidney of male rats. These changes could be due to the generation of ROS, causing damage to membrane and cell compartments. Co-administration of zinc had a protective effect and alleviated the harmful impact of fipronil. These outcome show that administration of Zn may be usfule, easy and economical to protect human especialy workers in agriculture sector against phenyl pyrazole insecticide toxic effects. Also, a great attention should be taken during the application of FPN in agriculture and household sectors to avoid its deleterious effects on farm animals, home applications and occupationally exposed humans.

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REFERENCES

1. Tingle, C.C.D., J.A. Rother, C.F. Dewhurst, S. Lauer and W.J. King, 2003. Fipronil: environmental fate, ecotoxicology and human health concerns. Rev. Environ. Contam. Toxicol., 176: 1-66.
2. Jennings, K.A., T.D. Canerdy, R.J. Keller, B.H. Atieh, R.B. Doss and R.C. Gupta, 2002. Human exposure to fipronil from dogs treated with Frontline. Vet. Hum. Toxicol., 44: 301-303.
3. Mansour, S.A. and A.T.H. Mossa, 2010. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pestic. Biochem. Physiol., 96: 14-23.
4. Mossa, A.T.H., T.M. Heikal, M. Belaiba, E.G. Raoelison, H. Ferhout and J. Bouajila, 2015. Antioxidant activity and hepatoprotective potential of *Cedrelopsis grevei* on cypermethrin induced oxidative stress and liver damage in male mice. BMC Complement. Altern. Med., Vol. 15. 10.1186/s12906-015-0740-2
5. Badgujar, P.C., G.A. Chandratre, N.N. Pawar, A.G. Telang and N.P. Kurade, 2016. Fipronil induced oxidative stress involves alterations in SOD1 and catalase gene expression in male mice liver: Protection by vitamins E and C. Environ. Toxicol., 31: 1147-1158.
6. Assayed, M.E., A.A. Khalaf and H.A. Salem, 2010. Protective effects of garlic extract and vitamin C against *in vivo* cypermethrin-induced cytogenetic damage in rat bone-marrow. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 702: 1-7.
7. Mossa, A.T.H., E.S. Swelam and S.M.M. Mohafrasha, 2015. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. Toxicol. Rep., 2: 775-784.
8. Mossa, A.T.H., A.A. Refaie and A. Ramadan, 2011. Effect of exposure to mixture of four organophosphate insecticides at no observed adverse effect level dose on rat liver: The protective role of vitamin C. Res. J. Environ. Toxic., 5: 323-335.
9. Yousef, M.I., F.M. El-Demerdash and K.S. Al-Salhen, 2003. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J. Environ. Sci. Health B, 38: 463-478.
10. Powell, S.R., 2000. The antioxidant properties of zinc. J. Nutr., 130: 1447S-1454S.
11. Parrat, M.O., M.J. Richard, J.C. Beani and A. Favier, 1997. Involvement of zinc in intracellular oxidant/antioxidant balance. Biol. Trace Elemenet Res., 60: 187-204.
12. Goel, A., V. Dani and D.K. Dhawan, 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chem. Biol. Interact., 156: 131-140.
13. Nishikimi, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.
14. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
15. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
16. Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.
17. Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.
18. Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta, 90: 37-43.
19. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
20. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
21. Sharma, D. and G.K. Sangha, 2014. Triazophos induced oxidative stress and histomorphological changes in liver and kidney of female albino rats. Pestic. Biochem. Physiol., 110: 71-80.
22. Al-Othman, A.M., K.S. Al-Numair, G.E. El-Desoky, K. Yusuf, Z.A. Al Othman, M.A. Aboul-Soud and J.P. Giesy, 2011. Protection of α-tocopherol and selenium against acute effects of malathion on liver and kidney of rats. Afr. J. Pharm. Pharmacol., 5: 1263-1271.
23. Sayeed, I., S. Parvez, S. Pandey, B. Bin-Hafeez, R. Haque and S. Raisuddin, 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicol. Environ. Saf., 56: 295-301.
24. Mossa, A.T.H., F.M. Ibrahim, S.M. Mohafrash, D.H. Abou Baker and S. El Gengaihi, 2015. Protective effect of ethanolic extract of grape pomace against the adverse effects of cypermethrin on weanling female rats. Evid. Complement. Altern. Med., Vol. 2015. 10.1155/2015/381919
25. Elgengaihi, S., A.T.H. Mossa, A.A. Refaie and D. Aboubaker, 2016. Hepatoprotective efficacy of *Cichorium intybus* L. extract against carbon tetrachloride-induced liver damage in rats. J. Dietary Suppl., 13: 570-584.
26. Kehrer, J.P., 1993. Free radicals as mediators of tissue injury and disease. Crit. Rev. Toxicol., 23: 21-48.

27. Banerjee, B.D., V. Seth, A. Bhattacharya, S.T. Pasha and A.K. Chakraborty, 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol. Lett., 107: 33-47.
28. Tukhtaev, K.R., S.K. Tulemetov, N.B. Zokirova, N.K. Tukhtaev and M.R. Tillabaev *et al*., 2013. Prolonged exposure of low doses of fipronil causes oxidative stress in pregnant rats and their offspring. Int. J. Toxicol., 10: 1-11.
29. Mansour, S.A. and A.T.H. Mossa, 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pestic. Biochem. Physiol., 93: 34-39.
30. Mossa, A.T.H., T.M. Heikal, S.M.M. Mohafrash and A.A. Refaie, 2015. Antioxidant potential and hepatoprotective activity of *Origanum majorana* leaves extract against oxidative damage and hepatotoxicity induced by pirimiphos-methyl in male mice. J. Applied Sci., 15: 69-79.
31. Hayes, J.D. and D.J. Pulford, 1995. The glutathione S-transferase supergene family: Regulation of gst and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol., 30: 445-600.
32. Margarido, T.C.S., A.A. Felicio, D.D.C. Rossa-Feres and E.A. de Almeida, 2013. Biochemical biomarkers in *Scinax fuscovarius* tadpoles exposed to a commercial formulation of the pesticide fipronil. Mar. Environ. Res., 91: 61-67.
33. Mossa, A.T.H., T.M. Heikal and E.A.A. Omara, 2014. Liver damage associated with exposure to aspirin and diazinon in male rats and the ameliorative effect of selenium. Biomed. Aging Pathol., 4: 137-145.
34. Yousef, M.I., T.I. Awad and E.H. Mohamed, 2006. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. Toxicology, 227: 240-247.
35. Fukino, H., M. Hirai, Y.M. Hsueh, S. Moriyasu and Y. Yamane, 1986. Mechanism of protection by zinc against mercuric chloride toxicity in rats: Effects of zinc and mercury on glutathionine metabolism. J. Toxicol. Environ. Health, 19: 75-89.
36. Seagrave, J.C., R.A. Tobey and C.E. Hildebrand, 1983. Zinc effects on glutathione metabolism relationship to zinc-induced protection from alkylating agents. Biochem. Pharmacol., 32: 3017-3021.
37. Prasad, A.S., 1993. Zinc and Enzymes. In: Biochemistry of Zinc, Prasad, A.S. (Ed.). Plenum Press, New York, pp: 17-53.
38. Prasad, A.S., 2008. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Exp. Gerontol., 43: 370-377.
39. Chavapil, M., J.N. Ryan and C.F. Zukoski, 1982. Effect of zinc on lipid peroxidation in liver microcosms and mitochondria. Proc. Soc. Exp. Biol. Med., 141: 150-158.
40. Baiomy, A.A., F.H. Attia, M.M. Soliman and O. Makrum, 2015. Protective effect of ginger and zinc chloride mixture on the liver and kidney alterations induced by malathion toxicity. Int. J. Immunopathol. Pharmacol., 28: 122-128.
41. Radwan, E.M., 2001. Biological and biochemical effects of certain insecticides on the spiny bollworm, *Eariasinsulana* (Boisd). Ph.D. Thesis, Faculty of Science, Ain Shams University, Egypt.
42. Saleem, M.A., A.R. Shakoori and D. Mantle, 1998. Macromolecular and enzymatic abnormalities induced by a synthetic pyrethroid, ripcord (Cypermethrin) in adult beetles of a stored grain pest, *Tribolium castaneum* (Herbst.)(Coleoptera: Tenebrionidae). Arch. Insect Biochem. Physiol., 39: 144-154.
43. Margoshes, M. and B.L. Vallee, 1957. A cadmium protein from equine kidney cortex. J. Am. Chem. Soc., 79: 4813-4814.
44. Eaton, D.L., N.H. Stacey, K.L.Wong and C.D. Klaassen, 1980. Dose-response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome P-450. Toxicol. Applied Pharmacol., 55: 393-402.
45. Sharma, R.P. and M.P. Verma, 1980. Metal-binding proteins in bovine and porcine hepatic and renal tissues: Isolation and characterization. Am. J. Vet. Res., 41: 548-551.
46. Onosaka, S. and M.G. Cherian, 1981. The induced synthesis of metallothionein in various tissues of rat in response to metals. I. Effect of repeated injection of cadmium salts. Toxicology, 22: 91-101.
47. Masao, S., O. Atsushi, T. Koichiro, M. Hiroshi and N. Yasushi, 1989. Metallothionein-I accumulation in the rat lung following a single paraquat administration. Toxicol. Lett., 45: 41-47.
48. Sayed, M.A., 2001. Effect of imidacloprid, lead and their mixture on the hepatic esterase activity and metallothionein. J. Agric. Sci. Mansoura Univ., 26: 3187-3202.
49. Webb, M. and K. Cain, 1982. Functions of metallothionein. Biochem. Pharmacol., 31: 137-142.
50. Beggel, S., I. Werner, R.E. Connon and J.P. Geist, 2012. Impacts of the phenylpyrazole insecticide fipronil on larval fish: Time-series gene transcription responses in fathead minnow (*Pimephales promelas*) following short-term exposure. Sci. Total Environ., 426: 160-165.