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Synthetic Insecticide Fipronil Induced Over Gene Expression, DNA and Liver Damage in Female Rats: The Protective Role of Fish Oil

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Abstract

Synthetic insecticide fipronil (FPN) is widely spread worldwide for controlling insects in agriculture and public health sectors. Thus, this work aimed to study the molecular mechanisms of FPN induced hepatotoxicity and the protective role of fish oil in female rats. Female rats were allocated in four groups of rats exposed to FPN and/or fish oil and control. Oxidative stress markers i.e., lipid peroxidation (LPO), antioxidant enzymes, and liver function enzymes, butyryl cholinesterase (BuChE), histopathological lesions, DNA damage, expression alteration of apoptosis and inflammatory related genes in liver tissues were assessed. FPN treatment induced significant changes in the levels of LPO, liver function, BuChE, histopathological lesions, over-expression of tested genes and elevation in DNA damage. In contrary, treatment of FPN-exposed rats with fish oil improved significantly the negative effects of FPN on liver tissues. The molecular mechanisms of FPN might be due to the formation of reactive oxygen species (ROS) and change in the oxidant/antioxidant statues. The hepatoprotective effect of fish oil might be attributed to the increase in the antioxidant capacity, which prevented the ROS generation induced by FPN in female rats.

Keywords: Fipronil, Fish oil, Antioxidant activity, DNA damage, Gene expression, Liver Histopathology

1.Introduction

Pesticides formulations are mixture of active ingredient(s) and other inert components including solvent, wetting and emulsifying agents. The toxicity information of the active ingredients only is not sufficient to assess the undesirable health impacts of commercial pesticides. Chemical family of phenylpyrazole such as fipronil (FPN) is widely used to control insects on several crops [1]. FPN is effective insecticide against insects from orthoptera, lepidoptera and coleopteran [2]. Increase the exposure rate to different members of phenylpyrazole is a public health concerns globally. Numerous literatures reported that FPN insecticide is a powerful toxicant to several mammalian species and also humans [3,4]. Even exposure to low doses of FPN but for long time leads to induce harmful toxicity to pregnant rodents and their generation [5]. For that reason, the WHO assured the requirement needed for toxic hazard evaluation of the pesticide formulations [6].

The high co ncentrations of FPN in water and the soil due to its general use and high stability in the environment lead to high toxicity from such harmful pollutant on several organisms in the surroundings such as mammals, fish and humans [7]. Exposure to FPN at low doses induces adverse toxicity effects and organ dysfunctions mediated reactive oxygen species (ROS) production [8,9]. Endocrine disruption and adverse reproductive effects in female rats caused by FPN [10] is associated with decrease in the levels of glutathione and elevation in lipid peroxidation in buffalo calves [11] and activity alteration of CAT and SOD activities in the liver of *Cyprinus carpio* [12]. Moreover, FPN increased levels of hepatic enzymes and decreased total thyroxine in plasma of female rat [13].

Liver is the greatest sensitive and main target organ to toxic effect of pesticides [14]. Hepatic tissues are sensitive to oxidative stress of pesticides due to disturbed balance between the degree of the antioxidant capability and oxidative stress [14,15]. Earlier studies investigated the potential toxicity mechanism of the pesticides and found that they induced oxidative stress affecting enzymatic and non-enzymatic antioxidants in laboratory animals [14-16].

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According to a number of published reports toxicity of pesticides could be coincided with increase of radical species formation which leads to induce tissue damage including kidney, liver and brain tissues [17,18]. Oxidative stress exhibits when the free radical species generation be more than the capacity of antioxidant in the target cell. The defense system mediated by endogenous antioxidants neutralizes toxic impacts of ROS species by sending electrons to these species and therefore decreasing their damage. Antioxidant enzymes normally mediate protection of body cells against oxidative damage. However, it has been shown that non-enzymatic antioxidants such as reduced glutathione and total thiol and enzymatic antioxidants, such as CAT, SOD, GR, GPx, and GST might be significantly affected by continuous exposure of pesticides [19].

Currently, there is a developed demand for using natural products in medical application, in both developing and developed countries as a replacement for using synthetic drugs, which might have adverse effects. In current years, fish oil is gaining attention as source of potential pharmaceuticals Omega-3 fatty acids [20]. These omega -3 polyunsaturated fatty acids such as DHA and EPA have been found as important components responsible for protective effects of dietary fish oil [21]. Fish oil Omega-3 fatty acids deficiency is known for the onset of autoimmune disease in animal models as responsible for delaying their anti-inflammatory properties and the severity of arthritis in human [22]. Evidences also show that supplementation of dietetic fish oil is connected with inhibited response with inflammation in rheumatoid arthritis [23] and psoriasis patients [24]. Moreover, omega -3 polyunsaturated fatty acids found in rich amount in fish oil such as EPA and DHA has been shown to be protective against experimentally induced colon cancer in great number of studies [25-27]. It has also been stated that the intake of omega-3fatty acids destroys the so-called free radical induced diseases such as aging, cancer and atherosclerosis [28]. Furthermore, many studies showed that fish oil prevents cisplatin-induced hepatotoxicity [29].

Moreover, it has been indicated that elevation of antioxidant enzyme activity and reduction of ROS formation are considered as most benefit actions attributed to fish oil feeding in animals exposed to cyclophosphamide inducing oxidative stress [30]. There are limited data available, for the best of our knowledge, that have investigated the impact of formulated fipronil on the liver function, molecular biomarkers related to oxidant/antioxidant status, apoptotic and inflammatory genes as well as DNA damage in female rats' liver. Thus, the current study pointed to investigate the adverse impacts of sub-chronic administration of formulated fipronil on histopathological, biochemical and molecular biomarkers in female rats' hepatic tissues. Also, the current work was conducted to estimate the role of fish oil in modulating fipronil induced hepatotoxicity in rat.

2.Materials and methods

2.1. Chemicals and reagents

Fipronil (Insecto SC 5%) is a product of BASF Company (China) and manufactured by, Sinochem Group NingboTechnical Co., Ltd., China. Kits used for biochemical measurements were purchased from Biodiagnostic Company (Dokki, Giza, Egypt). The kits for reverse transcription and PCR reaction were purchased from Fermentas (Germany). SYBR Green Mix for qRT-PCR was obtained from Stratagene (USA). Fish oil was purchased from Alsafa pharmacy (Dokki, Giza, Egypt).

2.2. Animals

Female albino rats weighing 125±6.2 g [average] were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Giza, Egypt. Rats were kept in polypropylene cages (five rats in each). Diet was introduced to rats in special cups; all groups had free access to food and water all time for 4 weeks under standardized housing conditions (12h light/dark cycle, temperature (23±2◦C) and a relative humidity of 48% in the laboratory). The rats were adapted for 1 week before the start of the experiment. All the groups of rats were kept according to the guidelines and welfare regarding animal protection accepted by NRC Local Ethical Review Committee and was conducted in agreement with the "Guide for the Care and Use of Laboratory Animals" [31].

2.3. Experimental design

Rats were randomized into four groups, five rats in each group. Group1(G1): rats received water and served as a control. Group2(G2): rats received fish oil at 117.6 mg/kg b.wt. via oral route [27].Group3 (G3): animals received fipronil at a dose 9.7mg/kg b.wt. (1/10 of LD_{50} 97mg/kg b.wt.) via oral route for 28 consecutive days [32].Group4(G4): animals received fipronil and after 30 mints received fish oil at the dose mentioned above. Body weights were monitored weekly during the experimental period. All rats were noticed for any signs of toxicity or mortality throughout the experiment duration. Vehicle used for fipronil and fish oil and the rout of administration.

2.4. Blood samples and tissue preparation

At the end of the experiment, rats were fasted overnight and blood samples were collected by puncturing the retero-orbital venous plexus of the

animals with a fine sterilized glass capillary, then rats were sacrificed by cervical dislocation.. Blood samples were left to clot in clean dry tubes and centrifuged at 3000 rpm (600 x g) for 10 min at 4ºC to get the sera. Serum was stored at -20 ºC for further biochemical analyses. Liver was removed immediately after sacrificed, washed in saline and weighed. Liver small pieces of each animal was homogenized in 10% (w/v) ice cold 100 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm and then the supernatant was obtained and used for oxidative stress and antioxidant enzyme analyses. Other parts of liver were used for histopathology, gene expression and DNA damage analyses.

2.5. Serum biochemical parameters

2.5.1. Oxidative stress biomarkers in liver

Determination of antioxidant enzymes and lipid peroxidation (LPO) were performed according to the details given in Biodiagnostic kit's guidelines. Liver Superoxide dismutase (SOD) and catalase enzyme activities were determined according to the method of Nishikimi [33] and Abei [34] respectively. Liver glutathione-s-transferase was measured spectrophotometrically according to the method of Habig [35]. Lipid peroxidation was estimated by determining thiobarbituric acid reactive substances (TBARS) and was expressed in relations of malondialdehyde (MDA) content by a colorimetric technique according to Satoh [36]. Protein concentration was measured according to the method described by Gornal [37].

2.5.2. Serum liver biomarkers

Serum AST and ALT were measured according to the methods of Reitman and Frankel [38], butyryl cholinesterase BuCHE according to Knedel and Böttger [39], and ALP according to Young et al [40]. All biomarkers were performed according to the details given in Biodiagnostic kit's instructions (Biodiagnostic Company, Dokki, Giza, Egypt).

2.6. Expression of Caspase-3 (apoptotic gene), TNF-α, and IL-1β (inflammatory genes)

2.6.1. Isolation of total RNA

Total RNA was isolated from liver tissues of female rats using TRIzol® Reagent (Invitrogen, Germany) according to the manufacturer's instructions. One unit of RQ1 RNAse-free DNAse kit (Invitrogen, Germany) was used to digest DNA residues from isolated RNA and re-suspended in DEPC-treated water. Aliquots were used immediately for reverse transcription (RT); otherwise, they were stored at -80°C.

2.6.2. Reverse transcription (RT) reaction

Complete Poly(A)+ RNA isolated from liver tissues of female rats was reverse transcribed into cDNA in a total volume of 20 µl using RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, Germany). The RT reaction tubes were transferred to the thermocycler (Applied Biosystem, USA) and the reaction program was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with a denaturation step at 99°C for 5 min. The obtained cDNA was stored at -20°C or was used immediately for amplification through quantitative Real Time-polymerase chain reaction (qRT-PCR) [41].

2.6.3. Quantitative real time- PCR (qRT-PCR)

A StepOne Real-Time PCR System (Applied Biosystem, USA) was used to determine the liver cDNA copy number. PCR reactions were set up in 25 µl reaction mixtures according to the manufacturer's instructions of SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.). The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle was divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step (was carried out to obtain the melting curve) consisted of 71 cycles, which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. The sequences of specific primer of the genes used are listed in Table (1). The quantitative values of qRT-PCR of apoptosis and inflammatory genes were normalized on the bases of β-actin expression. The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta CT}$ method [42].

2.7. Comet Assay

Comet assay was performed referring to the protocol developed by Blasiak [43], with minor modifications. Liver tissues of female rats of each treatment were mixed with low-melting-point agarose (ratio of1:10v/v), then pipetted to precoated slides with normal-melting-point agarose. The slides were kept flat at 4°C for 30 min in dark environment. The third layer of low melting point agarose was then pipetted on slides, left to solidify at for 30 min 4°C. The slides were transferred to pre-chilled lysis solution, kept for 60min at 4°C. After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 min. Slides were subjected to an electrophoresis run at 0.8 V/cm, 300mAmps at 4°C for 30 min. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air-dried. Ethidium bromide was used for slides stain then and visualized by using Zeiss epifluorescence microscope (510–560 nm, barrier filter 590 nm) with a magnification of ×400. 100 cells

per animal were scored then analyzed with DNA damage analysis software (Comet Score, TriTek corp., Sumerduck, VA22742).

2.8. Histopathological studies

Liver tissues were cut and dehydrated in graded serial of alcohol and fixed in paraffin wax. Five micrometer thick pieces were cut and stained by hematoxylin and eosin (H&E). One slide was prepared for this organ; each slide contained ten field areas and two sections were examined for histopathological changes [44]. The examination was done using a light microscope (Olympus BX50) with a digital camera (Olympus E-410). The histopathological alterations in liver tissues were scored as follows: normal appearance (−), mild (+), moderate (++) and severe (+++).

2.9. Statistical analysis

All data values were expressed as means ± standard error (S.E.M). The data were analyzed with the Statistical Package for Social Sciences (SPSS 0.18 for windows). The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan's test for comparison between different treatment groups. Statistical significance was set at $P \leq 0.05$.

3.Results

3.1. Signs of toxicity

The results showed that no clinical signs such as diarrhea, hair loss, bloated abdomen and nasal hemorrhage of fipronil poisoning were detected among female rats of experimental groups. Furthermore, no mortality was showed throughout treatment duration.

3.2. Body weight gain

As shown in Figure 1 a statistically significant decrease in the body weight gain (%) was found in fipronil treated group compared to control group (43% vs 58%). Animals treated with fipronil showed significant increase in relative liver weight compared to control (3.26% versus 2.83%). Co-administration of fish oil + fipronil improved values to be relatively as control values.

3.3. Assessment of enzymatic and protein markers

The results revealed that the activity levels of AST, ALT and ALP were increased significantly after 28 days of exposure to 1/10 LD_{50} of fipronil when compared to the control group (Figure 2 A-C). Moreover, fipronil increased the levels of total protein (8.26 g/dl) compared to untreated one (6.91 g/dl) (Figure 2E). However, the activities of butyryl cholinesterase (2410.9 u/l) had significantly decreased compared to control group (3440.3u/l) (Figure 2D). Co-administration of fish oil with fipronil decreased significantly the activities of ALT, AST, and ALP (47.07, 44.58 u/ml and 49.50 u/l) compared with fipronil group (Figure 2A-C). These findings indicated that fipronil altered serum enzymatic biomarkers in a corresponding dose.

3.4. Determination of oxidative stress parameters

Levels of CAT, SOD, GST and lipid peroxidation assessed in hepatic tissues of female rats supplemented with fipronil for 4 weeks are summarized in Table 2. Rats exposed to fipronil showed significant decrease in the activity of SOD (5.03 vs 5.98 nmol/mg protein), CAT (36.47 vs 42.68 u/mg protein) and GST (79.7 vs 88.91 u/mg protein) in liver tissue while LPO level was significantly increased compared to control group (1.5 vs 0.89 nmol/g tissue). After administration of fish oil, the results showed improvement in the activities of CAT ,SOD and GST (40.08 and 5.68 u/mg protein and 87.08 nmol/mg protein respectively) in liver tissues compared with fipronil group values (Table 2). Also co-administration of fish oil with fipronil cause significant decrease in LPO level compared with fipronil group.

Table 1: Primer sequences of Caspase-3 (apoptotic gene), TNF-α, and IL-1β (inflammatory genes) used for qRT-PCR amplification

Gene	Primer sequence (5'–3')	References
Caspase-3	Forward: TGA GCA TTG ACA CAA TAC AC	[67]
	Reverse: AAG CCG AAA CTC TTC ATC	
TNF-α	Forward: ACT GAA CTT CGG GGT GAT TG	[68]
	Reverse: GCT TGG TGG TTT GCT ACG AC	
IL-1β	Forward: GCT AGT GTG TGA TGT TCC CAT TAG	[69]
	Reverse: CTT TTC CAT CTT CTT CTT TGG GTA	
β-actin	Forward: CAC GTG GGC CGC TCT AGG CAC CAA	[70]
	Reverse: CTC TTT GAT GTC ACG CAC GAT TTC	

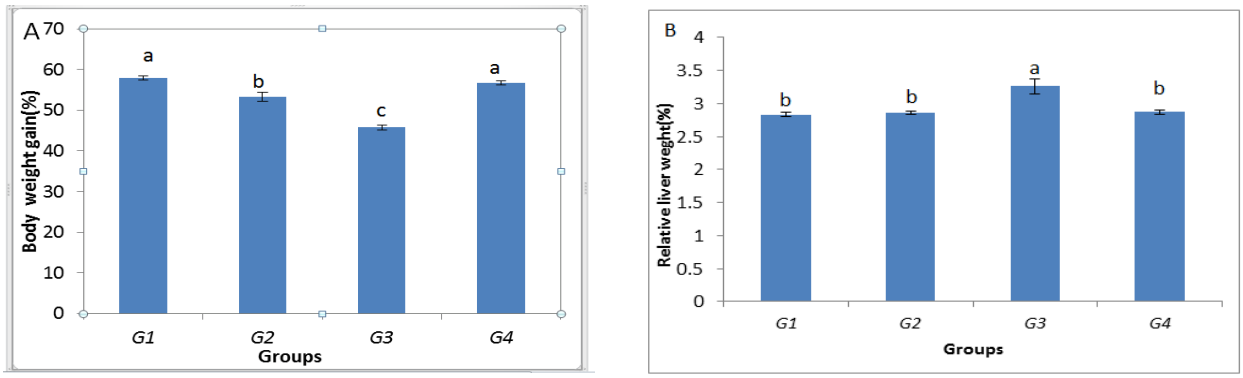

Figure 1: Effect of fish oil on fipronil induced alteration in body weight gain (%)(A)& Relative liver weight (%) (B)

Body weight gain (%) = [(final b .w t. – initial b .wt.)/ initial b .wt.] X 100; relative liver weight (%) (B) related to b. wt. = [liver weight /final body weight] X 100. Each value is a mean ± SE; [a b c] values are not sharing superscripts letters (a, b, c) differ significantly at $P \leq 0.05$.

3.5. Effect of fish oil on the gene expression alteration induced by fipronil

Expression of Caspase-3 (apoptotic gene), TNF-α, and IL-1β (inflammatory genes) quantified by qRT-PCR are summarized in Figures 3-5. The present study found high significant elevation (P<0.01) in the expression levels of Caspase-3 mRNA in female rats exposed to fipronil compared to those in control rats (Figure 3). However, treatment of female rats with fish oil alone showed low expression levels of Caspase-3 similar to the level of control female rats. Moreover, treatment of fipronil-rats with fish oil decreased significantly (P<0.05) the expression levels of Caspase-3 compared with those in the group exposed to fipronil.

Expression levels of IL-1β and TNF-α mRNAs in the liver tissues of female rats exposed to fipronil increased significantly (P<0.01) compared to those in control rats (Figures 4 and 5). In contrast, treatment of female rats with fish oil alone exhibited decreased in the expression levels of IL-1β and TNF-α to be relatively similar to the levels of control female rats. Moreover, treatment of fipronil-rats with fish oil showed a decline in the expression levels of TNF-α close to the levels of control female rats. Furthermore, treatment of fipronil-rats with fish oil decreased significantly (P<0.05) the expression levels of IL-1β compared with those of the group exposed to fipronil.

3.6. Fipronil induced DNA damage

DNA damage determined in the rats' genome was performed and summarized in Table 3. Treatment of female rats with fish oil showed low DNA damage which was relatively close to that in control group. Conversely, fipronil treatment increased significantly (P<0.01) the rate of DNA damage in comparison with control group. Moreover, treatment of fipronil-exposed rats with fish oil reduced significantly (P<0.05) the rate of DNA damage compared with that in female rats exposed only to fipronil.

3.7. Histopathological observations

Fipronil induced histopathological changes in liver of female rat as in fig (6 C) , showing the hepatic architecture with dilated congested sinusoids (S) with inflammation around the central vein (C.V) , activated kupffer cells, the portal area (P.A) have inflammatory cells around their vessels (H&E200x). Co- administration of fish oil with fipronil improved these alterations (Fig 6A- D).

Table 2: Effect of fipronil on liver antioxidant enzymes and lipid peroxide activities in the liver tissue of female rats

Groups	Catalase (u/mg protein)	Glutathione –s-transferase (u/mg protein)	Superoxide dismutase (µmol /mg protein)	Lipid peroxidation (nmol/mg protein)
G1	42.68±0.56[a]	88.91±1.04[a]	5.98±0.10[a]	0.89.±0.017[b]
G2	42.66±0.46 [a]	88.08±0.97[a]	5.88±0.09[a]	0.85±0.010 [b]
G3	36.47±0.88 [c]	79.70±1.01[b]	5.03±0.11[b]	1.5±0.12[a]
G4	40.08±0.44[b]	87.08±1.21 [a]	5.68±0.20[a]	1.03±0.05[b]

Value expressed as a mean of 5 animals ± S.E.; [a, b, c, d] values differ significantly at P< 0.05.Control group(G1),fish oil treated group (G2),fipronil-treated group(G3) and fish oil plus fipronil-treated group(G4).

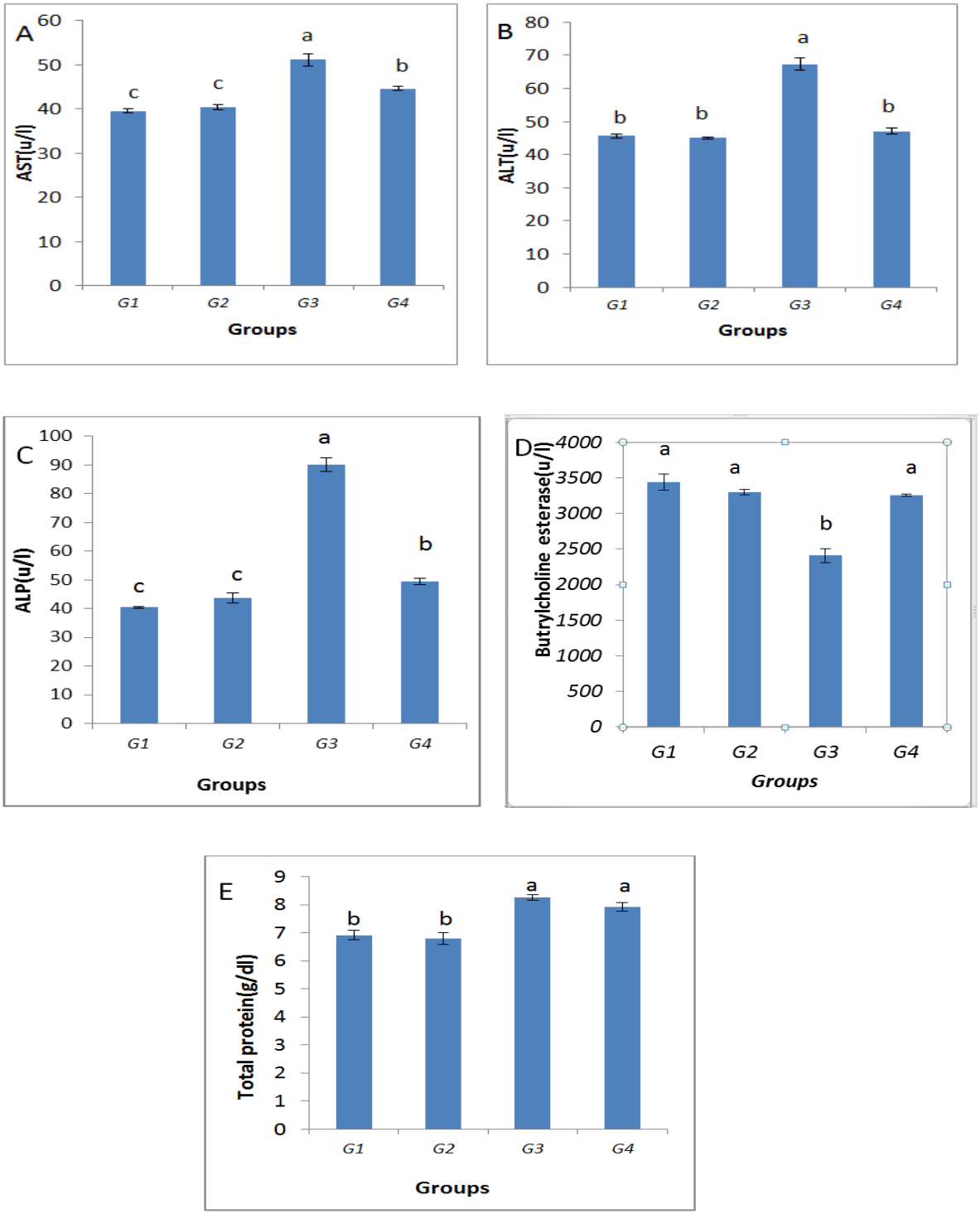

Figure 2: Effect of fish oil on fipronil induced alteration in aspartate AST (A), ALT(B), ALP (C), BuChE (D) total protein (E) concentration in the sera of female rats.

Each value is a mean of 5 rats± SE; [a b c] values are not sharing superscripts letters differ significantly at $P \leq 0.05$, (G1) control, (G2) fish oil group, (G3) fipronil group (G4) fipronil + Fish oil group.

Table 3: Rate of DNA damage in liver tissues of female rats after exposure to Fipronil and treated with fish oil using comet assay.

Groups	No of samples	No. of cells		Class**				DNA damaged cells % (Mean ± SEM)
		Analyzed*	Comets	0	1	2	3	
G1	**5**	500	34	466	23	11	0	6.8±0.37[c]
G2	**5**	500	33	467	25	8	0	6.6±0.39[c]
G3	**5**	500	106	394	42	38	26	21.2±0.79[a]
G4	**5**	500	66	434	35	14	17	13.2±0.58[b]

¥: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of cells analyzed were 100 per an animal. Each value is a mean of ± SE; [a b c] values are not sharing superscripts letters (a, b, c) differ significantly at p ≤ 0.05.

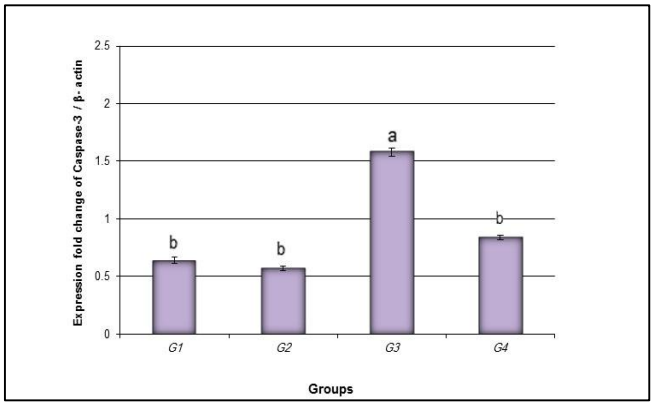

Figure 3: Expression levels of Caspase-3 mRNA in liver tissues of female rats exposed to Fipronil and treated with fish oil determined by quantitative RT-PCR.

Data are presented as mean ± S.E.M for at least five rats in each group. [a, b:] Mean values in the same column with different superscript differ significantly ($p < 0.05$).

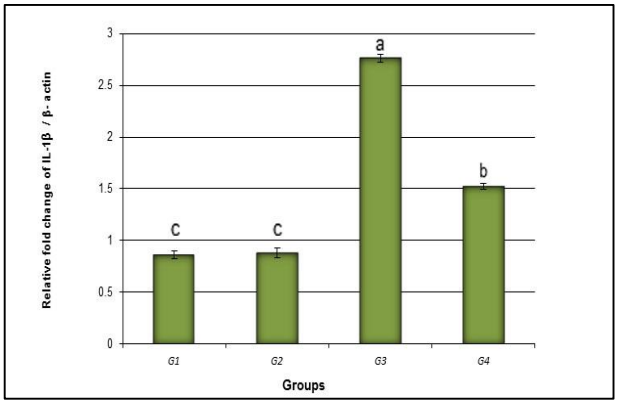

Figure 4: Expression levels of IL-1β mRNA in liver tissues of female rats exposed to Fipronil and treated with fish oil determined by quantitative RT-PCR.

Data are presented as mean ± S.E.M for at least five rats in each group. [a, b, c:] Mean values in the same column with different superscript differ significantly ($p < 0.05$).

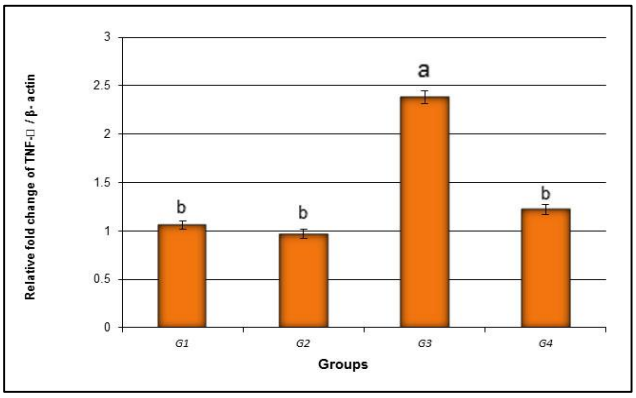

Figure 5: Expression levels of TNF-α mRNA in liver tissues of female rats exposed to Fipronil and treated with fish oil determined by quantitative RT-PCR.

Data are presented as mean ± S.E.M for at least five rats in each group. [a, b:] Mean values in the same column with different superscript differ significantly ($P < 0.05$).

Table 4: Histopathological changes in the liver of female rats exposed to fipronil and the protective effect of fish oil, based on scoring severity of injury.

Observation	G1	G2	G3	G4
Inflammatory cells in the portal area	-	-	++	-
Congestion in hepatic sinusoids	-	-	++	+
Diffuse kupffer proliferation	-	-	+	-

Normal (−),mild (+), moderate (++), severe(+++).

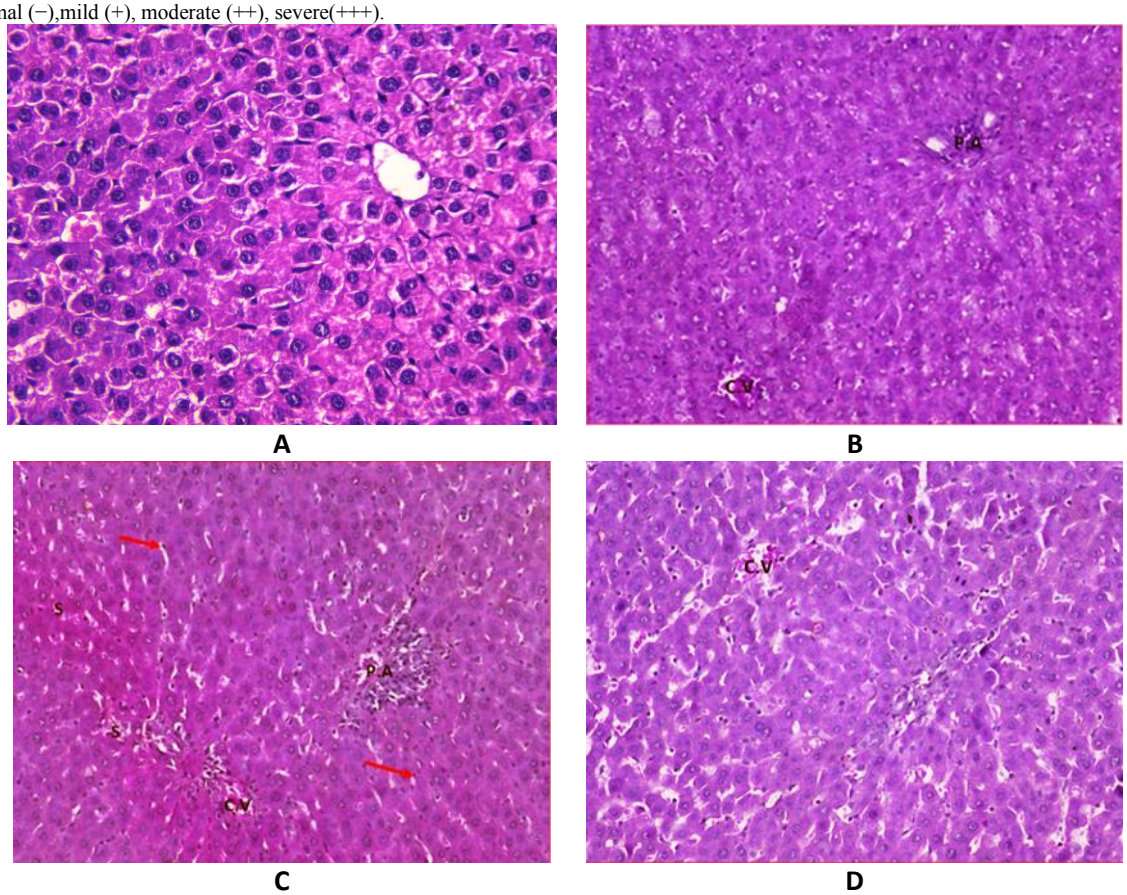

Figure 6: (A-D):Photomicrography of liver sections (H and E, 200X) showing normal liver tissue in (A) Control group with normal hepatocytes and central vein (thin arrow), (B) fish oil treated group showing normal hepatic architecture as centrally located the central vein (C.V), (C) fipronil group showing dilated congested hepatic sinusoids with inflammation around the central vein, activated Kupffer cells, the portal area have inflammatory cells around their vessels .(D) fipronil+fish oil group showing the normal hepatic architecture with dilated congested hepatic sinusoids, normally looking hepatocytes and central vein (C.V) (H&E200x).

4. Discussion

The present work exhibited that relative liver weight was increased significantly in rats exposed to fipronil compared to control rats. This elevation in relative liver weight could be resulted from several toxicological impacts due to exposure to FPN which induce oxidative stress mainly in liver tissues [14,45,46]. Fipronil treatment caused an increase in liver weight of rats due to increase the ROS generation induced inflammation as reported by US Environmental Protection Agency [47].

The present results indicated that exposure to 1/10LD_{50} of fipronil caused liver damage in the form of enzymatic and protein levels changes. Several serum biomarkers were altered such as AST, ALT and ALP as well as total protein levels were increased, while butyryl cholinesterase levels were decreased in rats exposed to FPN. It has been well known that catabolism and biosynthesis of amino acids are mediated by enzymatic biomarkers activity such as ALT and AST. These enzymes are responsible for several biological processes such as detoxification and metabolism pathways as well as energetic macromolecules biosynthesis for a range of cellular crucial functions [48,49]. Additionally, the increase in ALT and AST are considered as specific signs for hepatic damage [50]. The levels changes of ALT and AST enzymes could be attributed to disorder and dysfunction of liver biosynthesis of the macromolecules with alteration in the membrane permeability of hepatic cells [51]. In the same line with our findings, Ola *et al.* [52] reported that male

buffalo calves supplemented with 0.5 mg $kg^{-1}day^{-1}$ fipronil for 3 weeks produced significant elevation in plasma enzymes such as AST and acid phosphatase as well as increase in blood glucose and total plasma proteins.

The current study indicated that fipronil caused significant decrease in butyryl cholinesterase (BuChE). This effect could be due to the liver damage and disrupt biosynthesis of enzyme. These findings are in agreement with other studies [53-55].

The current study found that FPN induced significant alteration in the expression levels of apoptotic (caspase-3) and inflammatory (TNF-α, and IL-1β) genes and increase the rate of DNA damage as well as increase the histopathological lesions in liver tissues. In same line, Badgujar *et al*. [56] found that fipronil exposure in both male and female mice caused significant increase in the frequency of micronuclei (MN) and DNA damage. Moreover, Badgujar *et al.* [57] reported that fipronil caused significant decrease in the hepatic mRNA expression of SOD and CAT genes as well as an increase in the lipid peroxidation (LPO). Moreover, they found that FPN decreased the activity of antioxidant enzymes; SOD, CAT and GST. Additionally, Badgujar *et al.* [57] showed severe fatty degeneration, focal necrosis in the hepatic parenchyma and vacuolation leading to hepatocellular necrosis which in same line with the current results.

The present work exhibited that fipronil supplementation induced oxidative stress in the liver cells as generation of LPO was increased. LPO elevation is well known to disorder cellular membranes integrity and associated with pathogenesis induction of various liver injuries [58,59]. So, LPO has used as biomarkers of pesticides caused oxidative stresses [60] and recommended as one of the main molecular mechanisms associated with toxicity mediated by pesticides exposure [61]. Increase the MDA levels as it represent the levels of lipid peroxidation in the hepatic cells of rats exposed to FPN might be attributed to increase ROS generation affecting antioxidant defense system [45]. Tukhtaev *et al*. [5] reported that exposure to low doses of fipronil for long time leads to induce harmful toxicity to pregnant rodents and their generation due to increase the levels of LPO in liver cells. Numerous studies showed that pesticides increased lipid peroxidation in animals [58,59,62]. Enzymatic and Non-enzymatic antioxidants are acting together to overcome the toxic impact of ROS generation in liver cells and enhancing pathway of protection toward oxidative stress [63]. Therefore, main role of FPN in liver toxicity is coincided with decrease levels of antioxidant enzymes and increase consequently the ROS generation inducing gene expression alteration, DNA damage and histopathological alterations. It is well known that, SOD enzyme enhances rapid dismutation of superoxide anion to less H_2O_2 molecules in which it is quick converted to H_2O and O_2 by activity of CAT enzyme [58,59]. The present study found that exposure of female rats to FPN decreased the activity levels of CAT and SOD in hepatic cells and consequently may be increased levels of free radicals induced oxidative damage in liver.

Fish oil is the rich sources of omega 3 and is subsequently considered as potent antioxidants. Omega-3-Fatty acids are consisting of the essential fatty acids, DHA and EPA. Both DHA and EPA fall into a larger category of polyunsaturated fatty acids (PUFAs). Omega 3-fatty acids have been presented to have beneficial effects against a number of different pathologies [64]. Several reports found that liver is affected by oxidative stress probably by amplifying the capacity of free radical chain reaction CAT [58,59]. However, natural products containing Omega-3-Fatty acids are enhancing the immune system to produce high levels of antioxidants preventing the free radical generation [65]. So, our findings found that treatment of female rats with FPN plus fish oil suppressed the role of FPN that induce ROS generation. Whereas, levels of antioxidant increased in the group of FPN + fish oil compared with the group of exposed to FPN alone.

The present study found that supplementation of female rats with fish oil mitigated the toxicity of fipronil promoted oxidative stress and decline related biomarkers alterations in the hepatic cells of female rats. The reduction in the levels of MAD by Omega-3 supplementation could be attributed to enhancement the antioxidant defense that prevented lipid peroxidation [66] and this action protect the cellular integrity, helps stabilize the reactive radicals and restrain the severity of fipronil.

5. Conclusion

Exposure to Fipronil induced a significant elevation in LPO and reduction in antioxidant levels of SOD, CAT and GST enzymes in the hepatic cells of female rats. These alterations might be attributed to ROS generation inducing damage to cell compartments and membrane inducing alteration in expression of apoptotic and inflammatory genes and DNA damage as well as increase the histopathological lesions. So, an excessive care should be recommended during FPN applications to avoid its harmful impacts. Moreover, co-supplementation of fish oil to the agricultural workers should be taken in consideration

to mitigate the potential harmful effects of FPN. These findings indicate that supplementation of fish oil seemed to be a hopeful products for safety against fipronil-enhanced oxidative stress and hepatotoxicity.

6-Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

7-Acknowledgments

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مبيد الحشرات فيبرونيل يستحث زيادة التعبير الجينى وتلف الحامض النووى والكبد فى اناث الفئران: الدور الوقائى لزيت السمك

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المستخلص:

المبيد الحشري الصناعي فيبرونيل (FPN) منتشر على نطاق واسع في جميع أنحاء العالم لمكافحة الحشرات في قطاعي الزراعة والصحة العامة. وبالتالي ، يهدف هذا العمل إلى دراسة الآليات الجزيئية للسمية الكبدية التي يسببها FPN والدور الوقائي لزيت السمك في إناث الفئران.

تم تخصيص إناث الفئران في أربع مجموعات من الفئران المعرضة لـ FPN و / أو زيت السمك الى جانب العينه الضابطه. تم تقييم علامات الإجهاد التأكسدي ، مثل بيروكسيد الدهون (LPO) ، والإنزيمات المضادة للأكسدة ، وإنزيمات وظائف الكبد ، والبوتريل كولينستراز (BuChE) ، والآفات النسيجية المرضية ، وتلف الحمض النووي ، وتغيير التعبير عن موت الخلايا المبرمج والجينات المرتبطة بالالتهابات في أنسجة الكبد. تسبب علاج FPN في حدوث تغييرات كبيرة في مستويات LPO ، ووظائف الكبد ، و BuChE ، والآفات النسيجية المرضية ، والإفراط في التعبير عن الجينات المختبرة ، وارتفاع تلف الحمض النووي.

على العكس من ذلك ، فإن علاج الفئران المعرضة للـ FPN بزيت السمك أدى إلى تحسن كبير في الآثار السلبية لـ FPN على أنسجة الكبد. قد تكون الآليات الجزيئية لـ FPN ناتجة عن تكوين أنواع الأكسجين التفاعلية (ROS) وتغيير تماثيل الأكسدة / مضادات الأكسدة. قد يُعزى التأثير الواقي للكبد لزيت السمك إلى زيادة قدرة مضادات الأكسدة التي تمنع توليد ROS الناجم عن FPN في إناث الجرذان.