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Fipronil Induced Hepatotoxicity, Genotoxicity, Oxidative Stress and the Possible Ameliorative Effect of Ginseng

Mohammad S. Al-Harbi[1*]

[1]*Department of Biology, Faculty of Science, Taif University, Taif 888, Saudi Arabia.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Fipronil (FPN) is a wide-spectrum insecticide and has been extensively used in agriculture since 1990s. The current study was deliberated to inspect the retrograde effects of exposure to the FPN on the hepatic tissues of male rats at concentration 10 mg/L in drinking water for 30 days. Serum aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activity and tumor necrosis factor –α (TNF-α) were significantly increased in FPN-treated rats. Oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reduced (GSH) were significantly decreased, while malondialdehyde level (MDA) was significantly increased in treating rats in a concentration dependent manner. From the obtained results, it can be concluded that FPN elicited lipid peroxidation, oxidative stress and liver injury in rats and ginseng has protective effect against lipid peroxidation induced by FPN as ginseng lowers the level of MDA and increased levels of SOD and CAT and also ginseng improved the immunological capacities by diminishing the level of TNF-α. These pathophysiological changes in liver tissues could be due to the toxic effect of FPN that related to a production of free radicals.

**Corresponding author: E-mail: msah90@hotmail.com;*

Keywords: Fipronil; rats liver; oxidative stress; ginseng.

ABBREVIATIONS

FPN: Fipronil; Gin: Ginseng; GSH: Reduced glutathione; GPx: Glutathione peroxidase; TNF-α: Tumour necrosis factor – Alpha; MDA: Malondialdehyde; CAT: Catalase.

1. INTRODUCTION

Fipronil is a phenylpyrazole insecticide that is heavily used to monitor insects in different grain crops and in public health directions [1]. It is more dynamic than organophosphate, carbamate and pyrethroids insecticides against different species of Lepidoptera, Orthoptera and Coleopteran [2,3].

Currently, exposure to phenylpyrazole pesticides is a universal public health and concerns are increased related to the proportional safety of these pesticides because of broad use, their toxicity, and releases into the environment. FPN is neurotoxic to insects and the primary mechanism of action refers to spasm leading to the death of insects at high doses [4].

FPN causes endocrine disturbance and adverse reproductive effects in female rats [5], increment in lipid peroxidation (LPO) and decrement in glutathione (GSH) at the dosage of 0.5 mg/kg/day for 98 days to calves [6] and modified SOD and CAT levels in the liver of Cyprinus carpio [7]. It increased hepatic enzymes in plasma of female rat [8], and caused human poisoning [9,10]. Liver is the most critical and main objective organ of pesticide toxicity and damage [11], Liver play a major role in the biotransformation of pesticides.

The susceptibility of hepatic tissues to the stress of pesticides is a function of the irritated balance between the oxidative stress and the antioxidant strength [11,12]. Previous studies reported that pesticides modify antioxidants and elicited oxidative stress in animals that was investigated as a potential mechanism of pesticide toxicity [13]. It has been reported that elongated exposure to low doses of fipronil initiates to oxidative stress in serum of pregnant rats and their offspring [14].

Pesticide formulations are complex mixtures that contain, besides the active ingredient(s), diverse other components, such as solvent, wetting, emulsifying agents, and additives; therefore the toxicity information on active ingredients alone is not appropriate to estimate the adverse health effects of commercial pesticides. Therefore, the WHO assert the necessity of evaluating toxic prohibition of the elicited pesticides [15].

Ginseng is a completely-known medicinal herb in traditional Asian medicine and is considered an adaptogen. Panax ginseng C.A. Meyer, which grows in China and Korea, has a variety of biological effectiveness that include anticarcinogenic, inflammatory effects, as well as cardiovascular safeguard [16].

Ginseng is a herbaceous medicinal plants which has antioxidants, including polyphenolic compounds, flavonoids and triterpenoid saponins [17]. Preceding investigations showed that dry root extract of ginseng has immunomodulatory actions through its regulation of cytokine production, phagocytic actions of monocytes/ macrophages and dendritic cells, as well as activation of T and B lymphocytes [18].

Meanwhile, the usage of FPN has elevated extremely and information on adverse health effects is very restricted. To my knowledge, there are no published studies that have examined the effect of FPN on oxidative status and the liver function biomarkers in male rats and using Gin as ameliorative compound against the side effects induced by FPN. Therefore, this study endeavored to evaluate the adverse effects of exposure to formulated FPN on oxidant/ antioxidant status and liver biomarkers of male rats.

2. MATERIALS AND METHODS

Animals and management male albino rats weighing 150±5 g were provided by King Fahed Medical Research Center in Jeddah (Kingdom of Saudi Arabia). Rats were stayed in polypropylene cages, with free access pellet diet, water ad libitum, under standardized conditions (12 h light/dark cycle, temperature (23 ± 2℃). The rats were adapted for 1 week before the start of the experiment. All the rats were kept according to the guidelines regarding animal protection approved by the European Community Directive (86/609/EEC) and National rules on animal care have been followed. After 2 weeks of

acclimation, animals were randomly divided into four groups with 7 animals in each one.

2.1 Chemicals

Fipronil (Insecto SC 5%) is a product of BASF Company and manufactured by, Sinochem Group NingboTechnical Co., Ltd., China. Ginseng was obtained from Sinochem jiangsu company-China (Batch No. 2004060-1).The assay kits used for biochemical measurements of 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 dehydroge-nase (LDH, EC 1.1.1.27), superoxide dismutase (SOD, EC1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione perox-idase (GPx, EC 1.11.1.9), glutathione reduced (GSH), Malondialdhyde (MDA) were purchased from Quimica Clinica Aplicada S.A. Spain.All reagents were of the highest purity commercially available.

2.2 Animals and Experimental Design

Rats were randomly divided into four experimental groups. Group I, received dist. Water and served as a control. The group (II) received FPN in drinking water at concentration 10 mg/L [19] for 30 successive days. The concentration of FPN was calculated depending on the percentage of energetic ingredients of commercial formulation of FPN. Concentration of FPN was freshly prepared and body weights were monitored weekly during the experimental period. All rats were observed for signs of toxicity and mortality daily for 30 days. The concentration used in this study represent 2.0 mg/kg b.wt. of FPN, based on average water consumptions and body weights of treated rats. The group (III) received ginseng at dose 100 mg/kg/BW. This dose was chosen based on previous studies of Voces et al. [20]. The group (IV) administered FPN and then followed by Gin at the same doses.

2.3 Blood and Tissue Samples

At the end of the experimental period, rats were fasted overnight and blood samples were collected by puncturing the retro-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 4◦C using Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany) to obtain the sera. Serum samples were stored at −20℃ for further biochemical investigations, such as AST, ALT, ALP and LDH. Liver was removed immediately after sacrificing, cleaned in saline and weighed. The other portions of liver were 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℃, and then the supernatant was obtained and used for oxidative stress biomarkers studies (SOD, CAT, GPx, GSH, LPO) and TNF-α activity.

2.4 Serum Biochemical Parameters

2.4.1 Liver function biomarkers

Serum AST and ALT were determined according to the methods of Reitman and Frankel [21], ALP according to Young et al. [22], LDH as an indicator of necrotic cell death was determined according to Vassault [23].

2.4.2 TNF-α quantitation by ELISA

The levels of TNF-α in Liver tissue was quantitated using an enzyme-linked immunosorbent (ELISA) assay (Komabiotech INC. Rat TNF-alpha ELISA kit, USA) according to the manufacturer's instructions.

2.4.3 Oxidative stress biomarkers in liver

Determination of SOD, CAT, GPx, GSH and lipid peroxidation (MDA) were performed according to the principals below of different methods are given for each concerned biochemical parameter. SOD was determined according to the method of Nishikimi et al. [24]. The method based on the ability of SOD enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye and the absorbance was read at 560 nm for 5 min.

SOD activity was expressed as units/mg protein. CAT was determined according to the method of Abei [25]. The method is based on the decomposition of H_2O_2 by catalase. The sample containing catalase is incubated in the presence of a known concentration of H_2O_2. The absorbance is measured at 510 nm.

CAT activity was expressed as µmol/mg protein. GST was determined according to Habig et al. [26]. 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 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 units/mg protein. The MDA values were expressed as nmoles of MDA/g protein. Protein concentration in homogenate was determined according to the method described by Lowry et al. [27].

2.5 Single Cell Gel Electrophoresis (SCGE) (Comet Assay)

Liver pieces of the treated and control groups were placed into a small Petri dish with an ice-cold mincing solution (Ca^{2+}- and Mg^{2+}-free HBSS containing 20 mM EDTA and 10% DMSO). The viability of the cells of the examined organs was indirectly determined by analyzing the comet images after electrophoresis. The samples were cut into smaller pieces, using a disposable microtome razor blade, and the solution was aspirated. Then, a fresh mincing solution was added and the samples were minced again to finer pieces. Resulting cell suspensions were collected and filtered (100 µm nylon meshes). All samples were stocked on ice in appropriate conditions to avoid light until the comet assay procedures.

Immediately before analysis, the DNA was stained with 50 µl of 20 µg/mL ethidium bromide. The slides were examined with a 40X objective lens with epi-illuminated fluorescence microscopy (Olympus-Bx60, excitation filter: 515-560 nm; barrier filter: 590 nm) attached to a color CCD video camera and connected to an image analysis system (Comet II, Perspective Instruments, UK). The Comets were analyzed by a visual scoring method and computerized image analysis [28]. To quantify DNA damage, tail length (TL), tail DNA (%) (TDNA) and tail moment (TM) were analyzed using Comet Assay Project Software (CAPS), generally, 50-100 randomly selected cells are analyzed per sample.

2.6 Statistical Analyses

Data were collected, arranged and reported as mean ± SEM of seven groups (each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS version 15.0. The statistical method was One way ANOVA test, and if significant differences between means were found, Duncan's multiple range test according to Snedecor and Cochran to estimate the effect of different treated groups (Snedecor GW, Cochran) [29].

3. RESULTS

3.1 Signs of Toxicity

No mortality manifested during the experimental period. Signs of toxicity included a change in activity and abnormal walk and other markers were observed in rats exposed to concentration 10 mg/L of FPN from the second week either alone or combined with Gin and all the signs were recorded as shown in Table 1.

3.2 Serum Biochemical Parameters and TNF-α Activity

The activity of serum enzymes; AST, ALT, ALP and LDH recorded significant increment after 30 days of exposure to 10 mg/L of FPN as compared to the control group (Table 2) and (Fig.1). On the other hand, In Gin treated group the activities of AST, ALP and LDH had no significant changes as compared to a normal control group. Meanwhile, the group treated with combinations of FPN and Gin afforded significant decrease in ALT, AST, ALP and LDH levels as compared to a normal control group and this elicited significant ameliorative results of liver enzymes as compared to the FPN treated group only. The results revealed that FPN changed serum biomarkers and Gin reduced these changes. Meanwhile, TNF-α activity was significantly increased in FPN treated group while it is significantly reduced in FPN and Gin combined group. There is no significant change was recorded in Gin treated group alone.

3.3 Oxidative Stress Biomarkers

As recorded in Table 3 and Fig. 2 (a,b and c) oxidative stress markers; SOD, CAT, GPx and MDA were specified in hepatic tissues of male rats exposed to 10 mg/L of FPN for 30 days. Rats exposed to 10 mg/L of FPN (group II) showed significant changes in the activity of GPx and MDA levels in liver tissue and SOD, CAT activities. In group IV, rats exposed to 10 mg/L of FPN in combination with Gin showed a significant elevation in oxidative stress biomarkers (SOD, CAT and GPx) were recorded

in liver while affording significant reduction on the MDA level as compared to a normal control group. The present results revealed that FPN caused statistically significant changes

Table 1. Signs of toxicity of fipronil alone and combined with ginseng on male rats

Findings	Groups	
	FPN group	FPN+Gin group
Change of the activity	++++	—+
Abdominal gait	++++	++–
Diarrhea	++++	—+
Myosis	+++-	++–
Increased urination	++++	++–
Diaphoresis	—+	—+
Nose and eye bleeding	++–	-------
Salivation	++–	—+
Death	-------	-------

------- *absence of the change in the animals of the studied group.*
++++ *a change which was often found in all the studied animals of a group.*
+++- *a change which was observed in almost all the studied animals of a group.*
++– *a change not so often observed in all animals of a group.*
—+ *a change which was rare within a group*

Table 2. Effect of fipronil and/or ginseng and their combinations on liver function parameters and TNF-α activity of male rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)
1-Control group	52.36±2.36[d]	30.62±1.54[c]	60.98±2.96[c]	115.87±4.63[c]
2- FPN group	99.65±3.21[a]	114.59±2.10[a]	103.65±4.25[a]	298.66±3.65[a]
3-Gin group	54.36±2.69[cd]	30.64±1.63[c]	61.02±3.65[c]	112.36±2.10[c]
4-FPN+Gin group	67.52±1.63[b]	71.63±2.69[b]	78.69±2.69[b]	202.47±1.96[b]

Values within the same column in each category carrying different letters are significant (P≤0.05) using Duncan's multiple range test, where the highest mean value has symbol a and decreasing in value was assigned alphabetically

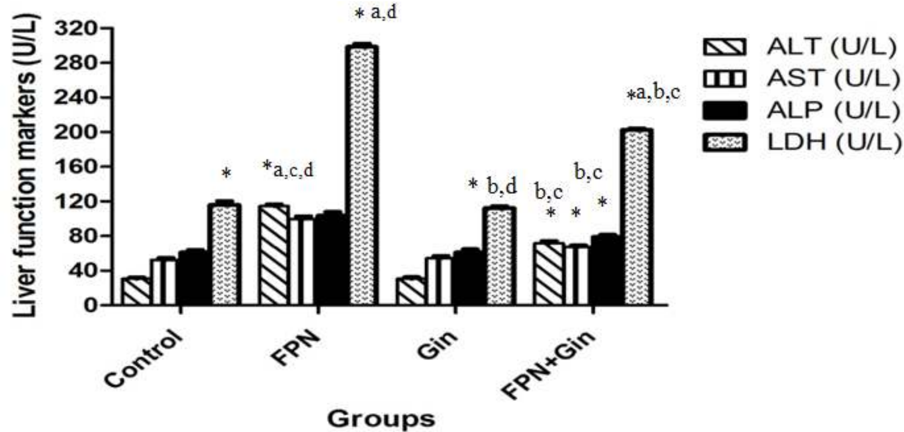


Fig. 1. Effect of of FPN (10 mg/kg wt) and/or gin (100 mg/kg wt) and their combination on liver enzyme biomarkers

**: Significant difference at* p<0.05., [a] Significant difference as compared to control group*
[b] significant as compared to FPN, [c] significant difference as compared to the group of Gin (100 mg/Kg),
[d] Significant difference as compared to the combined group of FPN and Gin

in oxidative stress biomarkers in the liver homogenates while the combination of FPN with Gin ameliorated antioxidant biomarkers.

3.4 Comet Assay

Comet images of cells derived from liver of the rat of the control group which showed intact nuclei and normal round cell without a tail (Fig. 3A). The FPN-treated group showed damaged DNA strand breaks and damage nuclei and the higher degree of damage with appearance of more than one apoptotic cells with large tail and small head (Fig. 3B). Gin treated group showed intact nuclei with undamaged DNA in a supercoiled state (Fig. 3C). FPN + Gin treated group which showed more percent of intact cells with undamaged DNA and less numbers of comet cell. The measurement all are cited in Table 4 related to the diameters of DNA tail length and percent of apoptosis.

4. DISCUSSION

No mortality was recorded in rats exposed to the FPN at concentration 10 mg/L for 30 days. While the clinical markers related to FPN-treatment including a change in gait were recorded from the second week as it was abnormal. The prospect for FPN to afforded specific neurotoxicity as previously reported [30], rats [31] and human [10].

Fipronil is insecticide that is extremely used to control insects in different grain crops [1]. It is more efficient than organophosphate, carbamate and pyrethroids insecticides against several species of insects [2-3]. So using ginseng daily can reduce the hazardous effects of FPN which is used on important crops like grain crops which are extensively used and thus minimize the oxidative stress effects induced extensively by FPN.

The results of the current study proposed that exposure to 10 mg/L of FPN elicited liver damage in treated rats compared to control as proved by increases in serum marker enzymes AST, ALT, ALP and LDH. Liver enzymes in serum e.g. AST, ALT, ALP and LDH are mainly used in the estimation of hepatic damage. Transaminases (AST and ALT) play a vital role in amino acids catabolism and biosynthesis. They are responsible for detoxifying, metabolism and biosynthesis of energetic macromolecules for different fundamental functions [32,33] and used as specific markers for liver damage [34].

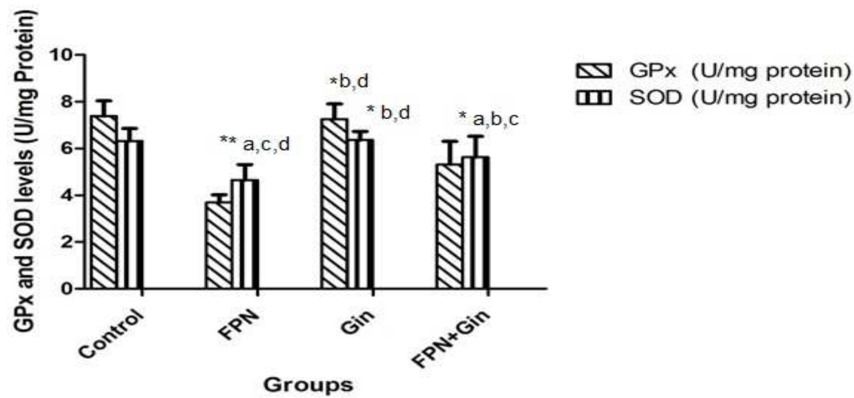
The increase in these enzymes may be due to liver dysfunction and biosynthesis of these enzymes with modification in the liver membrane permeability takes place [35]. In the current study, the elevation in LDH activity in serum of rat administrated FPN may be due to the hepatocellular necrosis and leakage of the hepatic enzymes [36]. In accordance with obtaining results, Oral administration of FPN at dose 0.5 mg kg−1day−1for 21days afforded significant increases in plasma LDH, AST, acid phosphatase, total plasma proteins and blood glucose in male calves [37].

The results demonstrated that FPN treatment caused oxidative stress in the liver of male rats, which is evident from the generation of lipid peroxidation (LPO). LPO is known to trouble the integrity of cellular membranes in the pathogenesis of various liver and kidney prejudices [38,39]. Therefore, it has used as biomarkers of pesticides afforded oxidative stresses [40] and pointed out as one of the molecular mechanisms involved in pesticides-induced toxicity [41].

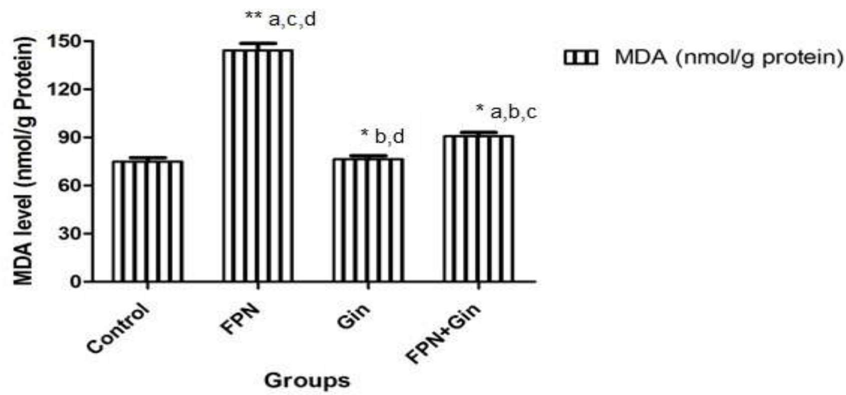
Table 3. Effect of fipronil and/or ginseng and their combinations on oxidative stress biomarkers in liver tissues of male rats

Groups	SOD (U/mg protein)	CAT (μmol/mg protein)	GPx (U/mg protein)	MDA (nmol/g protein)
1-Control group	6.32±0.53^{a}	13.57±0.68^{a}	7.39±0.65^{a}	74.98±2.33^{d}
2- FPN group	4.65±0.66^{c}	7.24±0.68^{c}	3.69±0.33^{c}	144.36±4.25^{a}
3-Gin group	6.36±0.37^{a}	13.68±1.66^{a}	7.25±0.65^{a}	76.25±2.36^{cd}
4-FPN+Gin group	5.63±0.89^{b}	10.36±1.32^{b}	5.32±0.98^{b}	90.67±2.41^{b}

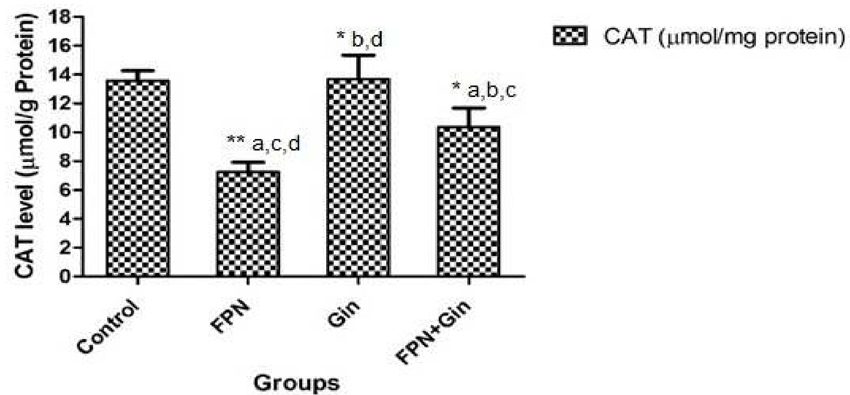
Values within the same column in each category carrying different letters are significant (P≤0.05) using Duncan's multiple range test, where the highest mean value has symbol a and decreasing in value was assigned alphabetically



(a) Effect of of FPN (10 mg/kg wt) and/or Gin (100 mg/kg wt) and their combination on SOD and GPX (U/mg) protein levels



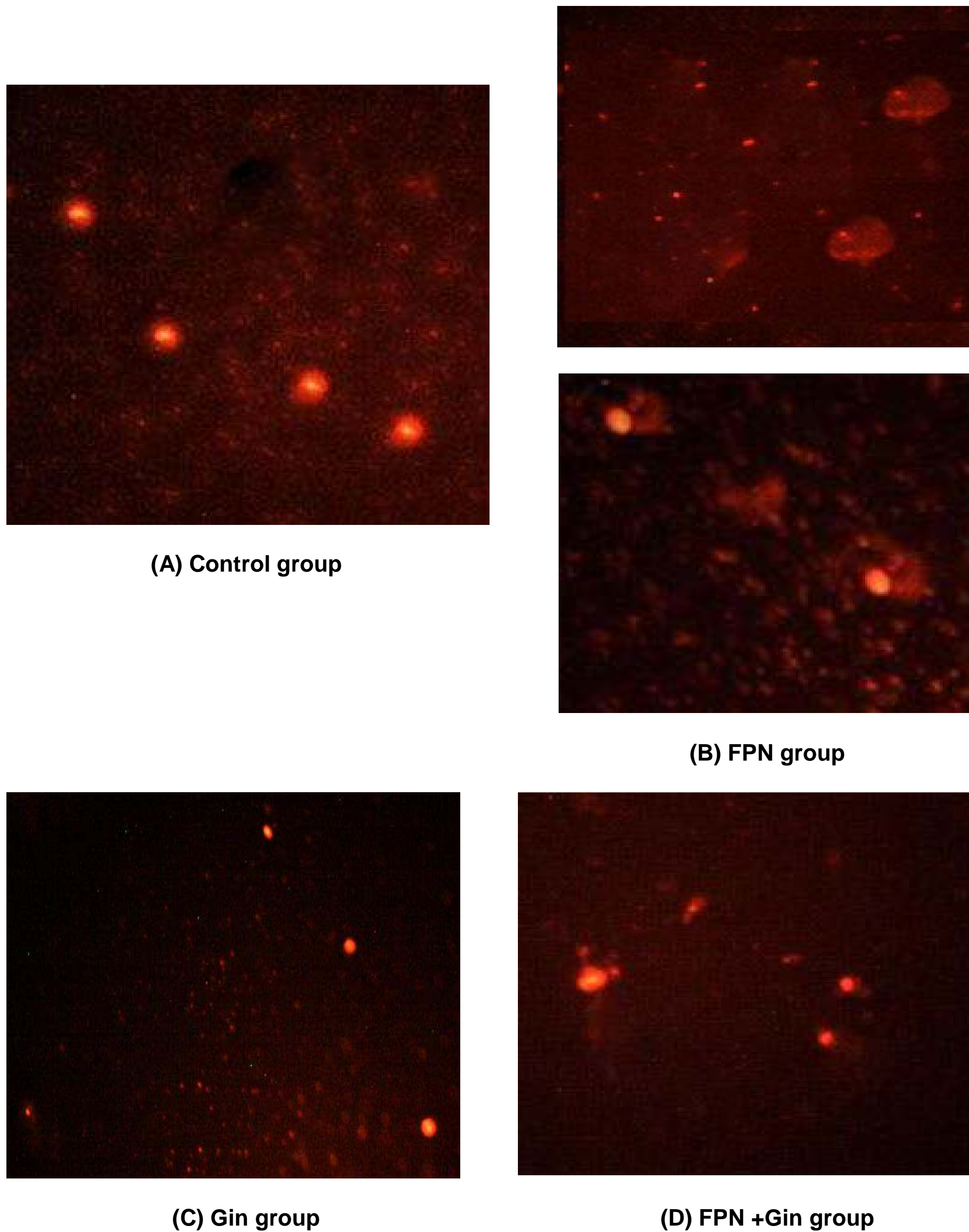
(b) Effect of of FPN (10 mg/kg wt) and/or Gin (100 mg/kg wt) and their combination on MDA (nmol/g) protein levels



(C) Effect of of FPN (10 mg/kg wt) and/or Gin (100 mg/kg wt) and their combination on CAT (μmol/mg) protein levels

Fig. 2. Effect of FPN (10 mg/kg wt) and/or Gin (100 mg/kg wt) and their combination on oxidative stress biomarkers

**: Significant difference at* $p<0.05$., [a] Significant difference as compared to control group*
[b] significant as compared to FPN, [c] significant difference as compared to the group of Gin (100 mg/Kg),
[d] Significant difference as compared to the combined group of FPN and Gin



(A) Control group

(B) FPN group

(C) Gin group

(D) FPN +Gin group

Fig. 3. Comet images of cells derived from liver of rat of group (A) control group which showed intact nuclei and normal round cell without tail, group (B) FPN treated group which showed damaged DNA strand breaks and damage nuclei as the cell contains a head like comet and with tail appear as hallow area and the higher degree of damage with appearance of more than one apoptotic cells with large tail and small head and the relaxed loops of damaged DNA extend to the anode to form a comet-shaped structure. (C) Gin treated group showed intact nuclei with undamaged DNA in a supercoiled state (D) FPN+Gin treated group which showed more percent of intact cells with undamged DNA and less numbers of comet cell

Table 4. The effect on oxidative DNA damage level (tail length, DNA% and tail moment) and apoptotic cell population (apoptosis %) in liver of rats treated with FPN and Gin and their combinations

Group	Tail length (px)	%DNA in tail	Tail moment (Units)	Apoptosis %
1- Control group	3.30±0.58	2.365±0.36	0.3643±0.05	8.634±1.21
2- FPN group	9.818±1.57 [a]	23.83±2.15 [a]	7.6336±1.68 [a]	86.160±4.65[a]
3- Gin group	3.166±0.57 [a,c]	2.124±0.87 [a,c]	0.3480±0.07 [a,c]	7.575±1.65[a,c]
4- FPN + Gin group	4.166±0.98 [b,c]	10.416±1.36 [b,c]	2.1672±0.54 [b,c]	40.583±5.25[b,c]

Values are expressed as means ± SE; n = 8 for each treatment group.
a Significant difference as compared to control group.
b Significant difference as compared to its related FPN group.
c Significant difference as compared to FPN with Gin group

Increased levels of malondialdehyde (MDA), a lipid peroxidation produced in the liver of FPN-treated rats may be due to increased production of reactive oxygen metabolites, especially hydroxyl radicals and convert antioxidant system [13]. Tukhtaev et al. [14] found that extended exposure to low doses of FPN increased LPO in liver of pregnant rats. Available studies indicated that insecticides increased LPO in animals [38,39].FPN-treatment afforded significant increase in oxidative stress by converting the enzyme activities associated with antioxidant mechanisms in the male rats' liver. It decreased the activity of antioxidant enzymes SOD, CAT and GPx level.

Thiobarbituric acid reactive substances are formed as a byproduct of lipid peroxidation which can be discovered by the TBARS assay using thiobarbituric acid as a marker. Because reactive oxygen species have excessively short half-lives, they are severe to measure directly. Instead, what can be measured are numerous products of the damage produced by oxidative stress, such as TBARS [42] and thus it is part of the analyses of stress markers not the only vital analyses but the presence of oxidative stress.

Assay of TBARS measures malondialdehyde (MDA), as well as malondialdehyde liberated from lipid hydroperoxides by the hydrolytic reactions [43]. MDA is one of the low-molecular-weight end products formed through the decomposition of primary and secondary lipid peroxidation products. However, only certain lipid peroxidation products elaborate MDA, and MDA is neither the sole end product of fatty peroxide formation and decomposition, nor a substance generated specially through lipid peroxidation.

Use of MDA analysis and/or the TBA test and interpretation of sample MDA content and TBA test response in studies of lipid peroxidation require caution correlative data from other indices of fatty peroxide formation [44].

Enzymatic and non-enzymatic antioxidants work together to prevent the effect of ROS in tissues and are active in the defense against oxidative cell injury by means of their being free radical scavengers [45]. Therefore, SOD, CAT and GPx are considered first defenses that protect cell macromolecules from oxidative damage. In this respect, SOD speed the dismutation of superoxide anion to less reactive molecule (H_2O_2) which is quickly converted to water and oxygen by CAT and GPx [46].

In the present study, the decreased in SOD, CAT and GPx levels in the liver in rats exposed to FPN could be due to excess production of O2•–which rapidly converted to H_2O_2 by SOD and to water by CAT and GPx. Previous studies reported that other pesticides leads to decline in SOD, CAT and GPx in liver of rats [39,46].

The change in oxidative stress, liver biomarkers in rats exposed to the FPN observed in this study are inconsistence with the past results obtained by Mossa et al. [47] as they revealed that FPN-treatment excess oxidative stress by altering the enzyme activities associated with antioxidant defense mechanisms in the liver and kidney of male rats. It caused decreases in the activity of antioxidant enzymes SOD, CAT, GPx and GST and level of GSH. Antioxidants work together to prohibit the effect of ROS in tissues and a reactive in the defense against oxidative stress by several ways of their being free radical scavengers [48]. Therefore, SOD, CAT and GPx are considered first defenses that conserve cell macromolecules from oxidative damage. In this respect, SOD accelerates the dismutation of superoxide anion to less reactive molecule (H_2O)

which is rapidly converted to water and oxygen by CAT and GPx [49,50].

On the same bases, Khan et al. [47] reported that exposure of FPN caused extreme ROS generation, lipid peroxidation and modification in mitochondrial membrane potential leading to apoptosis of spermatozoa in a dose dependent manner and confirmed the adverse effect of FPN on male reproductive functions which is mediated by oxidative stress, DNA damage and apoptosis in rat spermatozoa.

These results were confirmed by Badgujar et al. [51] who reported that fipronil afforded neuronal cell death has also been assigned to be mediated by ROS and oxidative stress. In their investigation, oxidative stress biomarkers were evaluated in the kidney and brain tissues of mice dosed fipronil orally for 28 consecutive days.

In agreement with the current study, a significant decrease in SOD activity was observed in liver of pregnant rats exposed to low doses of FPN [15], also SOD, CAT and GPx activity recorded decrement in diazinon in mice [52]. FPN can increase the production of reactive oxygen species in cells, which lead to increased oxidative stress [53]. It has been reported that FPN was responsible for oxidative stress in *C. carpio*, which was evident through alterations on antioxidant enzymes and increased lipid peroxidation levels [54].

GST play a vital role in metabolism, particularly in detoxification of xenobiotic e.g. pesticides and intracellular transport of metabolites. These enzymes catalyze the conjugation of reactive electrophiles with glutathione (GSH), consequently resulting in toxicological effects [55,56].

FPN reduced GST and GPx levels in frogs [57] and this is in agreement with the present study. The observations in the previous mentioned studies are in corroboration and support the obtained results.

4.1 Ginseng

Many in vitro and in vivo studies of herbal medicines have been conducted to identify natural products effective for treatment of many diseases and elimination of toxicity. Ginseng is one of the medical herbal plants that have multiple biochemical and pharmacological activities, including anti-inflammatory, anti-allergic, antidiabetic, anti-hyperlipidemic, antiosteoporosis, anti-cancer, antiangiogenesis, and rheumatoid arthritis. It has been used in the treatment of many diseases such as immune deficiency [58] as they showed that showed that the administration of either Chlorpyrifos or Profenofos elicited a non-significant increase in serum Tumor necrosis factor α (TNF- α) whereas, Propolis and/or ginseng afforded a non-significant decrease compared with either control group or groups given the insecticides alone, metabolic syndrome, hypertension, respiratory and hepatic dysfunction [59].

In vitro and *in vivo* studies proposed that ginseng reduces physical, chemical, and biological stress, while inducing general vitality, immune function and improving the central nervous system function. Also, ginseng root extracts have long been used to treat various diseases, including respiratory and hepatic dysfunction, hypertension, inflammation and diabetes in China, Korea and Japan [60].

To our knowledge, it is the first study reported the ameliorative effect of ginseng on oxidative stress and hepatotoxicity induced by fipronil.

In accordance with our data, previous study revealed the role of ginseng against the lipid peroxidation and nephrotoxicity induced by cisplatin as it is obvious that the nephrotoxicity of cisplatin involves reactive radicals. Thus the reasonable cellular protective agents against cisplatin toxicity involve antioxidant mechanisms to prevent GSH depletion and/or scavenge the intracellular reactive oxygen species as they showed that ginseng administration before cisplatin appeared to protect the kidneys against oxidative stress by reducing the oxidants such as TBARS, NO and XO, and enhancing the activities of antioxidant enzymes (SOD, GPX, CAT and GST) and reduced glutathione (GSH). Also, another confirmation to our data, ginsenosides may have improved the renal damage induced by CCl4 through scavenging of ROS and inhibition of the free radical generation [61]. Also, Ramesh et al. [62] reported that ginseng enhances the activities of SOD, CAT, GPx, GR and GST in kidneys, liver, heart and lungs of ginseng treated aged rats and this is greatly in agreement with the obtained results.

Ginseng possess antioxidant activities by enhancing antioxidant enzyme gene expressions which contribute to scavenging of ROS. Ginseng supplementation induces both antioxidant

enzyme activity and scavenging of free radicals [63].

The present results showed that ginseng co-treatment with FPN reduced its oxidative stress and increased the activities of antioxidant enzymes. Jin et al. [64] reported that ginseng reversed DNA fragmentation and apoptosis through the activation of antioxidant enzymes and suppression of the ROS generation and thus preserving the cell permeability.

Nevertheless, from the previous studies there are not enough data on the protective role of ginseng against FPN-induced hepatic toxicity. In the current study, there is an investigation of the effects of ginseng on oxidative stress in FPN-induced hepatic toxicity.

5. CONCLUSION

In view of the data of the present study, it can be concluded that FPN afforded liver damage and remarkable oxidative stress. FPN exposure produced highly increment in LPO and modification in antioxidant markers in liver tissues and elicited increment in liver enzymes. Therefore, the alteration in liver functions could be due to the production of ROS, which causing injury to membrane and all cell contents. On the other hand, the treatment of rats with Gin enhanced the antioxidant enzymes markers and ameliorated the liver function markers and the combination between Gin and FPN induced significant decline in liver function parameters and improved antioxidant enzymes of liver tissues.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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