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Sublethal effect of fipronil exposure on liver and kidney tissues with evaluation of the recovery ability of Japanese quail *(Coturnix japonica)*

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Abstract

Currently, there are limited toxicological data available for fipronil exposure effect on Japanese quail. The aim of the current study was to assess the toxicological effects on tissue histopathology and clinical biochemistry in a 15-day gavage study of fipronil as well as studying the physiological recovery of a 60-day depuration period after exposure in the Japanese quail. Relative liver and kidney weights in fipronil and recovery groups were non significantly changed than control. Histological changes in the liver and kidney were observed at fipronil group and recovery groups which showed restoration of the histoarchitecture particularly in an off dose 45 and 60 days groups. These changes were accompanied by clinical changes in the serum enzyme markers such as alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST) and lactate dehydrogenase (LDH). Clinical biochemistry markers for kidney were not altered in all groups, except creatinine level which showed a significant elevation in fipronil group only, although the presence of histological changes. Our results showed that fipronil exposure had a profound negative influence on the liver not in kidney injury indices and also on histological changes of Japanese quail liver and kidney tissues. Also, such changes were reversed after an off-dose period of 30 and 45 days.

Key words: Fipronil, Japanese quail, Phenypyrazole insecticides

Introduction

Fipronil is a member of the phenylpyrazole class of insecticides. It is more effective than organophosphate, carbamate and pyrethroids insecticides against resistant pests. The mechanism behind fipronil action is interfering with the passage of chloride ions through the GABA chloride channel having an effect on the neurotransmission that causes uncontrolled hyperexcitation and convulsions leading to death of insects at high doses[6)]. Fipronil is used to control ants, beetles, cockroaches, rootworms, and other insects. It is also being applied to control fleas and ticks on the domestic animals[4)]. Thus, fipronil is utilized in commercial (agricultural and veterinary purposes) and household applications.

Currently, concerns for potential adverse public health effects of fipronil have been raised that being classified as class C – possible

carcinogen. Besides, fipronil is highly toxic to many non-target organisms, such as honeybees, aquatic invertebrates, fish and birds[26], in which it can bind to mammalian GABAC and GABAA receptors[10,21], as well as its sulfone metabolite and fipronil- desulfinyl (a photodegradation product), were displayed to be more toxic to insects and non target species than the parent compound[7]. Several studies showed that fipronil caused endocrine interruption and adverse impacts in female rats reproduction[19]. It diminished total thyroxine, altered hepatic enzymes in female rat[13], central behavioral impacts in rats[23] and caused acute human poisoning[8,16]. Lyons[14] showed a substantial increase in thyroid cells, leading to tumor formation in male and female rats, suggesting that fipronil is highly carcinogenic. In addition, fipronil can have severe effects on fetal development and neonates following short- term exposure, such as learning disability, reflex reduction, infertility, as well as increased susceptibility to cancer and other diseases.

There are little data concerning the toxicological effects of fipronil in avian species and even less research documenting avian tissue morphological responses to fipronil ingestion. Thus, the toxic effect of oral dosing of fipronil following 15 days exposure and during a recovery period of 60 days in Japanese quail *(Coturnix japonica)* was assessed, on the basis of biochemical markers and histopathological investigation in liver and kidney tissues.

Materials and methods

Chemicals and biochemical reagents:

Fipronil insecticide was used as an available commercial formulation (Fipronil 20% Sc) provided by Yong- nong Bioscience Co, Ltd. China. All other chemicals and reagents were obtained from Sigma (St. Louis, MO, USA). All kits were purchased from Spinreact, SAU. Ltd. (Spain)

Animals:

Sixty mature male Japanese quail *(Coturnix coturnix Japonica)* weighing (200-230 gm) were obtained from Poultry farm, Faculty of Agriculture, Zagazig University, Egypt. Prior to the experiment, the quails were acclimatized for two weeks. Thereafter, the birds were held under a controlled photoperiod (12L: 12D, schedule of light– dark cycle), at 25 ± 2 °C with a relative humidity of 50 ± 5%. The birds were fed on standard commercial high protein diet (Al-Qahera Feeds, Egypt) and tap water was provided *ad libitum*. The experiments were done in conformity with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH), and the study protocol approved by the local authorities of Cairo University, Egypt.

Experimental groups and treatment:

Fipronil was dissolved in DMSO and used for treatment groups. The birds were randomly divided into two dosage groups, group 1 (n=10) kept as a control which was orally administered a DMSO vehicle only using gastric tube. Fipronil treated birds (n=50) were received fipronil orally at a dose of $^1/_5$ LD50 (LD50; 11.3 mg⁄kg [24]) daily for 15 days representing the fipronil group. Following exposure, 10 birds were sacrificed, the other 40 birds were kept for a recovery study, which subdivided into four equal groups; R_{15}, R_{30}, R_{45} and R_{60} which considered as a recovery groups after an off-dose period of 15, 30, 45 and 60 days, respectively. Throughout the study period, the quail was observed, and clinical signs were followed closely, as well as mortality incidence.

Body weight and relative organ weight:

Body weight of birds was recorded at the time of sacrificing, as well as kidney and liver weight was recorded after sacrifice for representing relative organ weight.

Blood sampling and tissue collection:

After treatment, the birds were bled on the days 0, 15, 30, 45 and 60 day post last dosage, as well as a control group. All blood samples were centrifuged directly at 3000 rpm for 10 min and serum was harvested and stored at -20°C for

further analysis. At necropsy of birds, kidney and liver specimens were sampled and fixed in 10% buffered neutral formalin solution for histopathological investigation.

Serum biochemical analysis:

Sera were used for estimation of serum liver injury biomarkers [Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH)], as well as renal injury biomarkers [Creatinine and Uric acid] according to manufacturer protocol.

Histopathological examination:

Specimens collected from kidney and liver were fixed in 10% neutral buffered formalin and were processed for histopathological examination. The processing involved dehydration in a series of alcohol concentrations (50%, 60%, 95% of absolute alcohol), clearing was done in xylene, infiltration, as well as embedding was done using molten paraffin wax. Five-micron thick paraffin sections were prepared, stained by H&E and examined microscopically using light microscope[3)].

Statistical analysis:

Data were expressed as mean ± SE. Statistical comparisons were performed by one way-ANOVA using the SPSS 16.0 computer program followed by Tukey's multiple comparison post-hoc test. A value of $p<0.05$ was considered as statistically significant.

Results

General observations and organ weight:

Quail administered fipronil orally via gastric intubation over a period of 15 days showed signs of decreased activity, anorexia, dullness and less responsive to disturbances. No mortality was observed in any of the groups. Severity of all signs, decreased with the prolongation of an off-dose periods. Also, The relative weight of the liver and kidneys in all groups showed non significant change from control throughout the experiment (Table 1)

Serum hepatic and renal injury biomarkers:

The activity of serum enzymes; AST, ALT and ALP was significantly elevated in fipronil administered and R_{15} groups with non significant change after 30, 45 and 60 days whereas their activity was reversed. On the other hand, concerning the activity of LDH, showed highly significant increases in fipronil and R_{15} and R_{30} groups in comparison to control. Besides, its level showed a non significant change in R_{45} and R_{60} group when compared with the control. Uric acid level was nearly similar to control in all experimental groups while creatinine levels elevated significantly in fipronil and non significantly in R_{15} and R_{30} groups which returned to control levels in other recovery groups (Table 1).

Histopathological observations:

Histologically, liver and kidneys of birds that were kept as control showed normal histological hepatic and renal architectures (Fig 1a and 2a). Hepatic lesions were more severe in fipronil and R_{15} groups represented by telangiectasia and engorgement of the hepatic sinusoids with blood (Fig 1b), severe centrilobular fatty degeneration and vacuolation of the hepatic cells (Fig 1c) as well as, presence of newly formed bile ductules in the portal areas (Fig1d). Focal aggregations of lymphocytes and heterophils were seen as well in the hepatic tissues of birds of the same groups. Liver sections of Japanese quail of R_{30} group showed marked restoration of the hepatic architecture with moderate to mild hydropic degeneration and vacuolation (Fig 1e), and focal aggregations of heterophils (Fig 1f). Birds of R_{45} group showed few focal aggregations of lymphocytes and heterophils in the hepatic tissues and individual cell necrosis of few hepatic cells (Fig 1g). While, R_{60} group showed mild degenerative changes; however, marked restoration of the normal hepatic tissue architecture was observed (Fig 1h). Kidney sections of Japanese quail of fipronil and R_{15} groups revealed inter-tubular edema, focal aggregations of macrophages with

fibroblasts proliferations (Fig 2b). Moreover, severe cystic dilatation of the collecting tubules with marked vacuolation of its lining epithelium was seen (Fig 2c). Renal lesions were moderate in The R_{30} group and consisted mainly of shrunken glomerular tufts and moderate to mild degenerative changes in the renal tubules (Fig 2d). In group R_{60}, a restoration of the normal histological architecture of the renal tissues was observed (Fig 2e).

Table 1: Effect of fipronil oral administration on Japanese quail liver and kidney relative weight (%) and tissues injury markers.

Parameters	Control	Fipronil	R_{15}	R_{30}	R_{45}	R_{60}
Liver relative weight	1.5±0.034	1.43±0.061	1.67±0.098	1.78±0.026	1.46±0.153	1.51±0.141
AST (IU/L)	148.6 ± 18.95^{cd}	301.9 ± 18.77^{a}	222.5 ± 8.54^{b}	177.46 ± 7.01^{bc}	153.44 ± 6.13^{cd}	114.36 ± 10.67^{d}
ALP (IU/L)	184.5 ± 7.18^{c}	303.4 ± 19.1^{a}	236.6 ± 12.19^{b}	208.92 ± 10.24^{bc}	187.36 ± 8.56^{c}	181.72 ± 3.7^{c}
ALT (IU/L)	$.7\pm6.25^{b}74$	154.14 ± 15.85^{a}	152.6 ± 5.90^{a}	117.56 ± 7.96^{ab}	108.09 ± 8.24^{b}	93.61 ± 9.98^{b}
LDH (IU/L)	78.8 ± 4.52^{d}	251.8 ± 19.47^{a}	202.83 ± 12.23^{b}	128.59 ± 8.31^{c}	94.09 ± 7.58^{cd}	95.60 ± 5.03^{cd}
Kidney relative weight	.707±0.040	0.72±0.040	0.68±0.04	0.65±0.029	0.69±0.03	0.74±0.023
Creatinine (mg/dL)	0.74 ± 0.02^{b}	1.02 ± 0.072^{a}	0.92 ± 0.049^{ab}	0.91 ± 0.055^{ab}	0.75 ± 0.045^{ab}	0.803 ± 0.073^{ab}
Uric acid (mg/dL)	3.44±0.77	5.01±0.165	3.42±0.450	4.05±0.53	3.73±0.174	4.096±0.56

IThe data are represented as mean ± SE (n=6) and evaluated by one-way analysis of variance (ANOVA) confirmed by Tukey' s test. Means within the same row (in each parameter) carrying different superscripts (a, b, c and d) are significantly different ($P < 0.05$).

Discussion

Fipronil is an essential member of the phenypyrazole group of insecticides and is widely utilized over different harvests and vegetables to control insects. Birds, animals and human beings at the danger of exposure to these insecticides. Liver and kidney are the most sensitive and principle target organs of pesticide toxicity and injury, whereas they assume a noteworthy role in the biotransformation of pesticides[15]. In the present study, fipronil was found to promote a potent hepatotoxic and mild nephrotoxic impact in treated birds compared to control. Fipronil elicited an increment in enzymatic biomarkers levels, including ALT and AST, ALP and LDH. Also, there was a significant increase in serum creatinine level. Liver enzymes in serum (AST, ALT, ALP and LDH) are principally applied in the assessment of hepatic disability. Transaminases (AST and ALT) play a significant role in amino acids catabolism and biosynthesis. They are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential processes[22] and used as a particular markers for liver injury. The increase in ALT and ALP enzymes may be referable to liver dysfunction and disturbance in the biosynthesis of these enzymes with modification in the integrity of the liver cell membrane[2], whereas the AST is a mitochondrial enzyme found in various organs and in plasma as well[7]. The elevated serum AST is obviously because of mitochondrial damage by reactive oxygen species (ROS) induced by fipronil[18]. Also, the rise in serum LDH activity of quail exposed to fipronil may be due to the hepatocelluar necrosis and leakage of the enzyme into the blood[9]. The elevation in the activity of these enzymes in serum may reflect pathologic changes in the liver.

Results revealed that fipronil administration caused an elevation in serum creatinine level which rapidly diminished in all recovery groups while uric acid proved a non significant increase. Generally, The increase of uric acid level may be by due to over production or the inability of excretion[17]. Also, the elevation of creatinine level in the serum is consequently a sign of impaired kidney function[11].

The injurious impacts of fipronil may result from

its generation of ROS that causes oxidative stress of various organs. Increased oxidative stress and lipid peroxidation are involved in the pathogenesis of pesticides-induced hepatic damage[1]. In this way, it has used as biomarkers of pesticides prompted oxidative stresses and proposed as one of the molecular mechanisms included in pesticides-induced toxicity[12]. Evidence of fipronil-induced injury has been exhibited by various studies, oral administration of fipronil induced significant increments in plasma LDH, AST, acid phosphatase, total plasma proteins and blood glucose in male buffalo calves[20]. Tukhtaev *et al.*[25] found that prolonged exposure to low doses of fipronil increased lipid peroxide in liver of pregnant rats and their progny.

Histopathological assessments are regularly utilized for identifying organ-specific effects related to chemical exposure[5]. The biochemical alterations observed in the current study are supported by histopathological findings. The histopathological evaluation showed various structural perturbations in fipronil group which showed an improvement all over the recovery period. This improvement is consistent with the biochemical results. These observations indicated marked changes in the general histoarchitecture of liver and kidney. These changes could be due to fipronil toxic impacts primarily as a response to the generation of ROS making damage to the various membrane components of the cell. Such observations on liver and kidney of fipronil treated group were comparable with previous study conducted in rat.

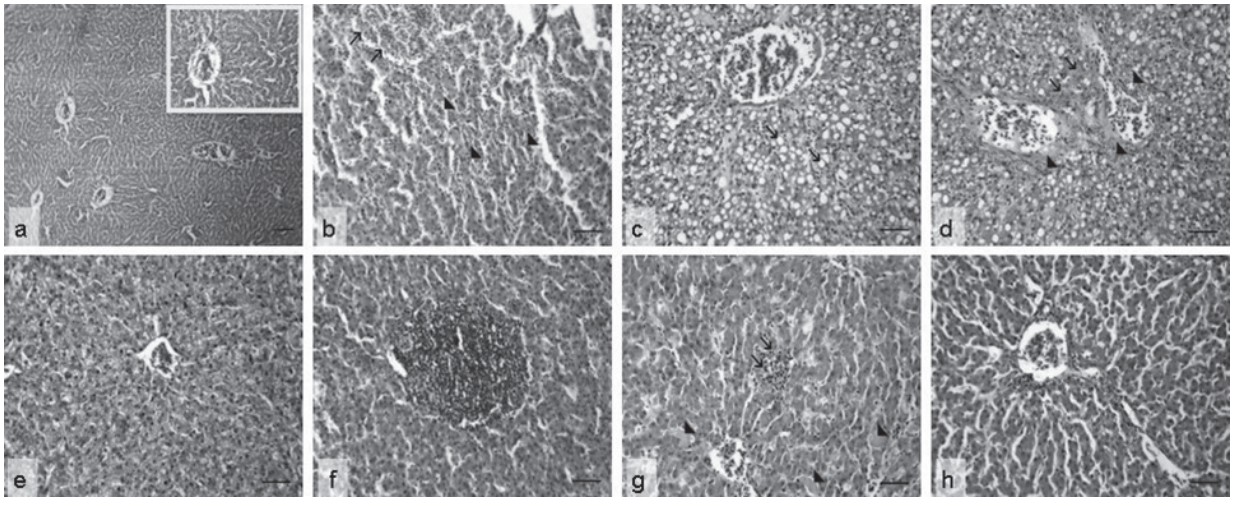

Fig. 1. Light microscopy of liver (Stain: H&E) from Japanese quail of the (a) control group, showing normal histological architecture of the hepatic tissues with intact cells and nuclei (Bar=100 μm, Inset; Bar=50 μm), (b) Fipronil group, showing telangiectasia (arrows) with plethro of the hepatic sinusoids (arrowheads). (c) R15 group showing diffuse severe centrilobular fatty degeneration and marked vacuolation (arrows), (d) R_{15} group showing fatty degeneration, vacuolation and leukocytic infiltrations (arrows) around the portal area with presence of newly forms bile ductules (arrowheads), (e) R_{30} group showing centrilobular hydropic degeneration and vacuolation of the hepatic cells, (f) R_{30} group showing focal aggregations of heterophils with pressure atrophy of the surrounding hepatic cells, (g) R_{45} group showing minute focal aggregations of lymphocytes and heterophils (arrows) with the presence of individual cell necrosis of few hepatic cells (arrowheads), (h) R_{60} group showing moderate disassociation of the hepatic cells with restoration of the normal histological hepatic architecture. (Bar=50 μm)

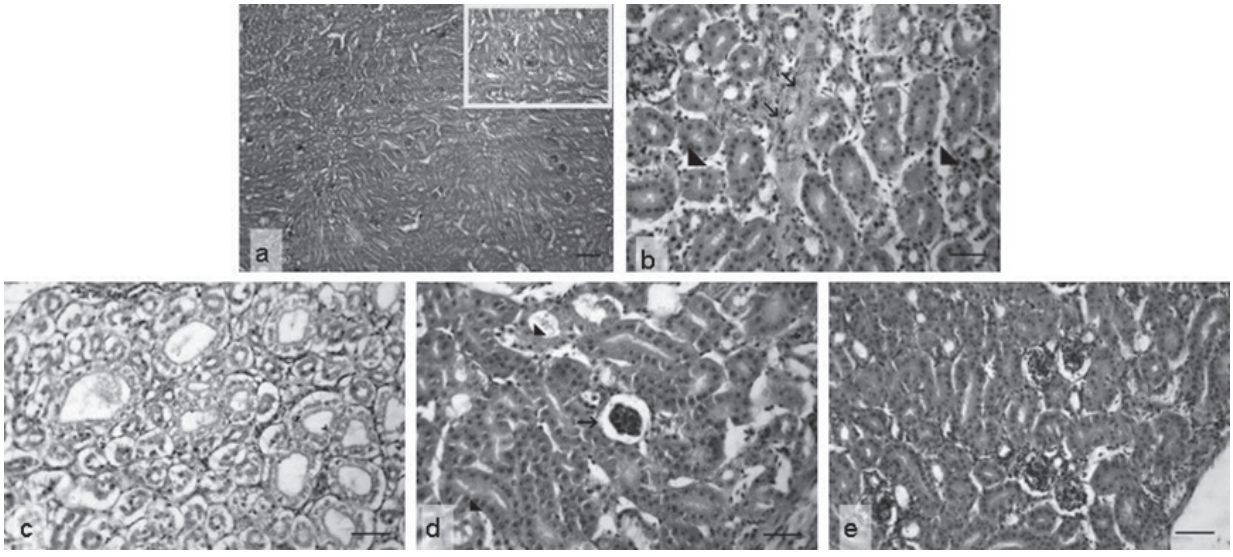

Fig. 2. Light microscopy of kidney (Stain: H&E) from Japanese quail of the (a) control group, showing normal histological architecture of the renal tissues with intact cells and nuclei (Bar=100 μm, Inset; Bar=50 μm), (b) Fipronil group, showing the inter-tubular edema (arrowheads), focal aggregations of macrophages, and fibroblasts proliferations (arrows), (c) R_{15} group showing severe cystic dilatation of the collecting tubules with marked vacuolation of its lining epithelium, (d) R_{30} group showing shrunken glomerulus (arrow) and mild degenerative changes of some renal tubules (arrowheads) with restoration of the normal renal histological architecture, (e) R_{60} group showing restoration of the normal histological architecture of the renal tissues. (Bar=50 μm) .

Conclusion

This study gives further evidence that fipronil is toxic to Japanese quail and causes changes in biochemical indices as well as histopathological changes which confirms a potentially hepatotoxic and somewhat nephrotoxic potential of fipronil. On the other hand, the recovery study showed a regeneration potential of organisms following an off dose of 30 and 45 days and confirmed that the responses of Japanese quail were in most indices reversible when exposure was terminated.

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