Histopathological Changes of Splanchnic Organs Induced by Fipronil Toxicity in White Leghorn Cockerels

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Abstract

Fipronil, introduced by Rhone-Plunac, is a member of phenyl pyrazoles which have herbicidal effects. Metabolic studies have showed that Fipronil can accumulate in fatty tissues, fish, fibre or food crops. Limited investigations have been carried on effect of Fipronil on animals and man. Therefore, present study was designed to study the effect of Fipronil toxicity in gross and histopathological parameters in White Leghorn Cockerels. Twenty, one-month old white leg horn male chickens were randomly divided into four groups of 5 birds each. Different doses of Fipronil were administered orally through feed at 1, 5 and 10 ppm levels for 100 days, daily. The tissue from liver, kidney, spleen, bursa of fabricius, testes and intestine were collected. The gross and histopathological changes were severe in group 3 in comparison to group 2 and 1. It may be concluded that even low dose of Fipronil has adverse effect on birds. These ill effects are dependent on dose and duration of exposure of Fipronil. Adequate measures should be taken to minimize the indiscriminate use of Fipronil, reducing poultry as well as human exposure to it and its further environmental contamination.

Key words: Insecticides, Gross Parameters, Histopathological Parameters, Fipronil, White Leghorn Cockerels, Kidney, Spleen, Bursa of Fabricius, Testes, Intestine

INTRODUCTION

The pesticides are classified as insecticides, fungicides, weedicides, herbicides, nematocides and rodenticides; of which insecticides constitutes 77% of the total pesticides used in different agricultural and animal husbandry practices and in public health operations. About 50% of food commodities are contaminated with pesticide residues in India. Livestock and poultry are frequently exposed to pesticide, drugs and environmental contaminants. Through contaminated feed, water, air insecticides are ingested and absorb in systemic circulation. They are metabolized and excreted through the animal body. However, some of the residue which remains inside body gets deposited in the various body tissues and is responsible for deleterious effect on various body organs. Pesticides are present in air, soil and water which leave their residue in food chain (Ram et al., 1987; Kaushik et al., 1991) causing deleterious effect on the health status of man and animals including poultry. Prolonged exposure to insecticides causes chronic neurological syndrome, malignant tumours, immunosuppressive action, teratogenic effect, abortion and decreased male fertility in experimental animals (Nafstad et al., 1983; Meeker et al., 2006; Yousef, 2010).

Fipronil was discovered and developed by Rhone-Poulenc between 1985-1987 and placed in the market in 1993. It is the member of new and relatively small class of pesticides, the phenyl pyrazoles, which are principally chemicals with a herbicidal effect. (Rhone Poulenc, 1995). Chemically, it is a (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[tri fluoromethyl] sulfanyl]-1H-pyrazole). It was registered as a pesticide in the United States in 1996 (Bobe et al., 1998). Fipronil or its metabolite noncompetitively inhibits GABA-induced ion influx by targeting the GABA-regulated chloride channels in insects. (Cole et al., 1993) Fipronil binding blocks the inhibitory action of GABA, leading to hyper excitation, and in appropriate concentrations even death (Bobe et al., 1998). Fipronil exhibits >500-fold selective toxicity to insects over mammals mainly because of differences in affinity in receptor binding between insect and man.

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mammalian receptor (Cole et al., 1993; Grant et al., 1998; Hainzl et al., 1998; Kamijima and Casida, 2000; Ratra et al., 2001; Zhao et al., 2005). Fipronil binds more tightly to the GABA_A receptor in insects than mammals.

Fipronil is sold commercially under various trade names including Icon, Regent, Ascend, Termidor, Chipko, Goliath, Chipko Choice and Adonis for agriculture use. It is available in a wide range of formulations including dispersible granule (WG), micro granule (GR), flowable solid (FS), soluble concentrate (SC) and ultra-low volume (ULV). It is also sold as veterinary product for tick, mite and flea control on pet and domestic stock under the trade names Frontline and Top spot. (Rhone Poulenc, 1996).

Fipronil is classified as a WHO Class II moderately hazardous pesticide. The U.S. EPA classified Fipronil as “Group C – possible human carcinogen” based on “increase in thyroid follicular cell tumours in both sexes of the rat”. There was no report regarding effect of Fipronil on the white leghorn chicken involving histopathological parameters. Therefore, present investigation was planned to study the effect of Fipronil at different dose rate on gross and histopathological parameters in white leghorn chickens.

MATERIALS AND METHODS

Twenty white leg horn male chickens of one-month old weighing about 150 to 200 gms were procured and kept at Instructional Poultry Farm Nagla, Pantnagar. The birds in poultry shed in deep litter system under standard management conditions and were randomly divided into four groups of 5 birds each. After one week of adaptation period, different doses of Fipronil were administered orally through feed at 1, 5 and 10 ppm levels to groups G1, G2 and G3 respectively for 100 days. The birds of group “C” served as control.

The birds were euthanized at 100 days. The tissue pieces from liver, kidney, spleen, bursa of fabricius, testes, and intestine was collected in 10% formal saline solution. Formalin fixed tissue were dehydrated in increasing strength of alcohol, cleared in xylene and embedded in paraffin blocks. Paraffin embedded sections were cut at 4-5 micrometer thickness and stained with haematoxylin and eosiin following the procedure of Culling (1975) and Lillie (1976).

RESULT

Pathological changes

1. Gross pathology

On post-mortem examination the experimental birds from group G1 revealed mild lesions. However, birds from group G2 and G3 severe congestion of intestine, liver, kidney and lung. Degeneration and necrosis of spleen, bursa, liver, and testis was highly appreciated in G2 and G3.

2. Histopathology

The tissue samples of kidneys, liver, spleen, intestine, testes and bursa of Fabricius from the sacrificed birds were collected for histopathological examination.

Kidney

Mild degeneration of tubular epithelium and glomerular tuft was seen in group G1. Degeneration of tubular epithelium, glomerular tuft and interstitial haemorrhage was seen in group G2. Severe necrosis of tubular epithelium, glomerular tuft, severe interstitial haemorrhage and cystic dilatation of tubules was seen in group G3. Changes observed in kidney of group G1, G2 and G3 at the end of study are shown in Plate No. 4.1, 4.2 and 4.3 respectively.

Liver

Various stage of degeneration, distortion of hepatic cord and dilatation of hepatic sinusoids was seen in Hepatocyte of liver of group G1. Degeneration, necrosis of hepatocyte, distortion of hepatic cord with haemorrhage was seen in liver of group G2. Extensive degeneration of hepatocyte, dilatation of hepatic sinusoids with extensive haemorrhage was seen in liver of group G3. Changes observed in liver of group G1, G2 and G3 at the end of study are shown in Plate No. 4.4, 4.5 and 4.6 respectively.

Spleen

Mild necrosis and paucity of cells was seen in spleen of group G1. Necrosis and paucity of cells with severe haemorrhage was seen in spleen of group G2. Severe necrosis and rarefaction of lymphoid cells with extensive haemorrhage was seen in spleen of group G3. Changes observed in spleen of group G1, G2 and G3 at the end of study are shown in Plate No. 4.7, 4.8 and 4.9 respectively.

Intestine

Mild desquamation and disruption of villi was seen in the intestine of group G1. Depletion of villi with congestion of mucosa was seen in the intestine of group G2. Severe disruption of villi and desquamation of epithelium was seen in intestine of group G3. Changes observed in intestine of group G3 at the end of study are shown in Plate No. 4.10 and 4.11 at different magnification.

Testis

Mild degeneration of seminiferous tubules of testis was seen group G1. Degeneration and necrosis of seminiferous tubules of testis was seen in group G2. Severe degeneration and necrosis of seminiferous tubules
of testis was seen in group G3. Changes observed in seminiferous tubules of testis of group G2 and G3 at the end of study are shown in Plate No. 4.12 and 4.13 respectively.

Bursa of Fabricius

Mild degeneration of lymphocyte in lymphoid follicles of bursa of Fabricius was seen in group G1. Degeneration and depletion of lymphocyte in lymphoid follicles bursa of Fabricius was seen in group G2. Severe degeneration and necrosis of lymphocyte in lymphoid follicle of bursa of Fabricius was observed in group G3. Changes observed in bursa of Fabricius of group G1, G2 and G3 at the end of study are shown in Plate No. 4.14, 4.15 and 4.16 respectively.

DISCUSSION

In tissue samples of liver and kidney the degenerative and necrotic changes were severe in G3 group in comparison to G2 group. Likewise, severe changes were reported in tissue samples of intestine, testis, spleen and Bursa of Fabricius in G3 group in comparison to G2 group. The histopathological changes were mild in G1 group in comparison to G2 group. These findings were consistent with the studies by other scientists demonstrating toxic effects of the pesticides. Peters conducted short term repeated dose on using Sprague Dwailey CD rats at 0, 25, 50, 100, 200 and 400 ppm Fipronil in the diet for 4 weeks. Study showed enlargement of the liver in some rats, mainly at 200 and 400 ppm, with generalised minimal hepatocyte enlargement in groups from 100 ppm. At the microscopic level, thyroid follicular hypertrophy (generally minimal, but moderate in some males at 200 and 400 ppm) was found in most treated animals at all doses, and not in controls. Peters et al. (1990)

A subchronic study was performed by Holmes (1991b) on rats using Fipronil in the diet at 0, 5, 30 or 300 ppm for 13 weeks. Fatty vacuolation of the liver was seen in all groups, including controls, but with the exception of an increased incidence of periaccinar vacuolation in 300 ppm males, was not considered treatment-related. Increased incidence of follicular cell hypertrophy and hyperplasia was seen in both sexes at 300 ppm.

In another study Holmes (1991c) administered Fipronil in gelatin capsules to groups of Beagle dogs (4/sex/group) at doses of 0.5, 2.0 and 10 mg/kg bw/d for 13 weeks. Microscopically, follicular and perifollicular atrophy of the mesenteric lymph nodes were observed in one dog at 10 mg/kg bw/d, and cortical atrophy of the thymus in another.

Broadmeadew (1993) conducted a study on Charles River CD-1 strain mice (72/sex/group) using Fipronil (purity 95.4%) in the diet at levels of 0, 0.1, 0.5, 10 or 30 ppm, equal to (males/females) 0/0, 0.01/0.01, 0.05/0.06, 1.2/1.2, 3.4/3.6 mg/kg bw/day. Twenty/sex/group was treated for 53 weeks (toxicity phase) and 52/sex/group for 78 weeks (oncogenicity phase). Statistically, a significant increase in the incidence of periaccinar microvesicular vacuolation was found in the livers of males treated at 10 or 30 ppm in both the toxicity and oncogenicity phases. In the oncogenicity phase, male mice treated at ≥10 ppm showed an increased incidence of chronic degenerative changes in the liver, including necrosis of occasional cells and apoptosis, increased ploidy, hypertrophy and degeneration of periaccinar hepatocytes, chronic inflammation and bile stasis. Male mice in the oncogenicity phase receiving 30 ppm showed a higher (but not statistically significant) incidence of malignant hepatocellular tumours. One hepatocellular carcinoma was also found in a 30 ppm mouse in the toxicity phase of the study.

Aughton (1993) performed chronic study on CD rats using Fipronil in the diet at levels of 0, 0.5, 1.5, 30 or 300 ppm. Study showed that in rats treated for 1 year and assigned to the reversibility period, six (4/15 from the 300 ppm group) had follicular cell tumours. When all rats assigned to the oncogenicity phase were considered together (irrespective of time of death), there was a significant increase in benign follicular cell adenomas for males (12/50) and females (8/50) receiving 300 ppm, and for males receiving 30 (3/50) or 1.5 (5/50) ppm. The incidence of increased follicular cell carcinomas was seen in male (5/50) and female (2/50) rats receiving 300 ppm compared with controls.

Fipronil (95.4% purity) was administered to two generations of Charles River CD strain rats by (King, 1992) at dietary levels of 0, 3, 30 or 300 ppm, respectively equal to (males/females) 0/0, 0.25/0.27, 2.5/2.7 and 26/28 mg/kg bw/day. Livers of 300 ppm females showed increased centriacinarian fatty vacuolation. Thyroids of 30 ppm males and of both males and females receiving 300 ppm showed follicular epithelial hypertrophy.

From the present study, it may be concluded that even a low dose of Fipronil has adverse effects on gross and histopathological features of different splanchnic organs of white leghorn cockerels. Thereby, it has been suggested that Fipronil at different toxic levels can damage the general health of birds. It can also affect the immune status of the birds due to its ill effects on immunological organs. These ill effects depend on dose and duration of Fipronil. Therefore, it is suggested that adequate measures should be taken to minimize the indiscriminate use of Fipronil to reduce further environmental contamination and the health hazard to poultry as well as humans.
Histopathological Changes of Splanchnic Organs Induced by Fipronil Toxicity in White Leghorn Cockerels

Plate 4.1 - Microphotograph of kidney showing mild degeneration of tubular epithelium and glomerular tuft in group G1 (H&E 200x).

Plate 4.2 - Microphotograph of kidney showing degeneration of tubular epithelium and glomerular tuft, interstitial haemorrhage in group G2 (H&E 200x).

Plate 4.3 - Microphotograph of kidney showing severe necrosis of tubular epithelium, glomerular tuft, severe interstitial haemorrhage and cystic dilatation of tubules in group G3 (H&E 200x).

Plate 4.4 - Microphotograph of liver showing hepatocytes at various stages of degeneration, distortion of hepatic cords and dilatation of hepatic sinusoids in group G1 (H&E 400x).

Plate 4.5 - Microphotograph of liver showing degeneration and necrosis of hepatocytes, distortion of hepatic cords with haemorrhage in group G2 (H&E 400x).

Plate 4.6 - Microphotograph of liver showing extensive degeneration of hepatocytes, dilatation of hepatic sinusoids with extensive haemorrhage in group G3 (H&E 400x).
Histopathological Changes of Splanchnic Organs Induced by Fipronil Toxicity in White Leghorn Cockerels

Plate 4.7 - Microphotograph of spleen showing mild necrosis and paucity of cells in group G1 (H&E 100x).

Plate 4.8 - Microphotograph of spleen showing necrosis and paucity of cells with severe haemorrhage in group G2 (H&E 100x).

Plate 4.9 - Microphotograph of spleen showing severe necrosis and rarefaction of lymphoid cells with extensive haemorrhage in group G3 (H&E 100x).

Plate 4.10 - Microphotograph of intestine showing disruption of villi and desquamation of villus epithelium in group G2 (H&E 100x).

Plate 4.11 - Microphotograph of intestine showing severe disruption of villi and desquamation of epithelium in group G3 (H&E 200x).

Plate 4.12 - Microphotograph of testis showing degeneration and necrosis of seminiferous tubules in group G2 (H&E 100x).
Histopathological Changes of Splanchnic Organs Induced by Fipronil Toxicity in White Leghorn Cockerels

Plate 4.13- Microphotograph of testis showing severe degeneration and necrosis of seminiferous tubules in group G3 (H&E 100x).

Plate 4.14- Microphotograph of bursa of Fabricius showing mild degeneration of lymphocytes in lymphoid follicles of in group G1 (H&E 200x).

Plate 4.15- Microphotograph of bursa of Fabricius showing degeneration and depletion of lymphocytes in lymphoid follicles in group G2 (H&E 200x).

Plate 4.16- Microphotograph of bursa of Fabricius showing extensive degeneration and necrosis of lymphoid follicle in group G3 (H&E 200x).

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