The Pharma Innovation Journal 2018; 7(4): 519-522

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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(4): 519-522

www.thepharmajournal.com
Received: 14-02-2018
Accepted: 16-03-2018

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Effect of spinosad and imidacloprid on histology of liver and kidney in male broilers and its amelioration with vitamin e and silymarin

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Abstract
Spinosad (SPD) and imidacloprid (IM) belongs to bacterial and neonicotinoid insecticides respectively widely used in grain crop cultivation throughout the globe and more in India which may find their way into feed channel of livestock. To study the toxic effects of SPD and IM and its combination the present experiment was designed to observe the toxicopathological effects in broilers and its amelioration with vitamin E (VE) and silymarin (SIL). A total of 120 male Cobb broilers were procured and divided into six groups consisting of 20 birds each with group 1 as control. The experiment was carried out for 4 weeks.

Histopathologically, group 2 (treated with IM) liver section revealed marked dilation and congestion of central vein and kidney section showed cystic dilation and degeneration of tubules. In group 3 birds (treated with SPD), the lesions found in liver sections were moderate to severe dilation of central vein, severe dilation of sinusoids, varied degrees of degeneration in hepatocytes and focal infiltration of mononuclear cells. Increased interstitial space, moderate to severe degeneration and necrosis of tubular epithelial cells were observed in kidneys. Histopathological changes of liver in group 4 (treated with IM+SPD) were severe congestion and dilation of central vein and sinusoidal spaces, necrosis and focal area of round cell infiltration, mild cystic dilation, intertubular haemorrhage and degeneration of tubules of kidneys. The microscopic changes in group 5 (treated with IM+SPD+VE) liver were mild vacuolar degeneration, moderate dilation of central vein and sinusoidal spaces. Kidneys revealed mild cystic dilation, proteinaceous casts, intertubular haemorrhages and focal to diffused round cell infiltration. In group 6 (treated with IM+SPD+SIL), the liver sections revealed a moderate to severe vacuolar degeneration, severe dilation of central vein and sinusoids, kidneys showed mild hypercellularity at areas of degenerated glomerulus and moderate to marked dilation of tubules. These results revealed that exposure of Imidacloprid, spinosad and its combination resulted in alterations in pathology of liver and kidney.

Keywords: effect of spinosad, imidacloprid, histology of, liver, kidney in male, vitamin e, silymarin

Introduction
The history of livestock and poultry in entire world coincides with the existence of human culture. Indian peasants especially in the state of Telangana mostly rely on backyard poultry as a source of livelihood. Earlier poultry was restricted to household only, but with abnormal increase in demand for poultry products like chicken and eggs were attributed to increasing population and improved scientific knowledge; now poultry has grown into an industry which is contributing to state economy.

Over the period of time insecticides and pesticides are being used extensively in the field of agriculture and veterinary science. However their indiscriminate use led to widespread concern because of their potential adverse effect on animal and human health (Al-saleh, 1994) [2].

Insecticides are broadly classified into organochlorides, organophosphates, carbamates, pyrethroids etc., and are being used throughout the world. Among all spinosad which is a bacterial insecticide introduced in market in 1997 has high efficacy, with broad insect pest spectrum, low mammalian toxicity, and a good environmental profile, which is having a unique feature of the insecticides that are currently used for the protection of grain products (Hertlain *et al.*,2011). Imidacloprid is a potent and most widely used insecticide introduced in the market in 1991 (Yamamoto and Casida 1999).

SPD is considered a natural product, and thus is approved for use in organic agriculture by numerous nations (Hertlain *et al.,* 2011). Imidacloprid is a potent hepatotoxic and nephrotoxic agent in rats and chicken (Arfat *et al.,* 2014 and Kammon *et al.,* 2010) [3, 8]. Both VE and SIL

have an antioxidant effects independently and when given together may enhance the immunoprotective and immunostimulatory properties of each other (Horvath *et al.*, 2001) [7].

Materials and methods

In the present experiment, a total of 120 day old male broiler chicks (Cobb strain) weighing between 32 -34 g were procured from a commercial hatchery. On arrival, the chicks were individually weighed, wing banded and divided into six groups of 20 each. The chicks were housed in battery brooders located at poultry experimental station (PES) and maintained under identical conditions throughout the course of experiment. The experiment was conducted with prior approval of the Institutional Animal Ethics Committee (IAEC).

The experimental design adopted for the present study is shown in Table 1.

Table 1

Group	No. of birds	Treatment
1	20	Control
2	20	Imidacloprid @ 50 PPM in feed
3	20	Spinosad @ 1000 PPM in feed
4	20	Imidacloprid @ 50 PPM + Spinosad @ 1000 PPM in feed
5	20	Imidacloprid @ 50 PPM + Spinosad @ 1000 PPM + Vitamin E @ 20 PPM in feed
6	20	Imidacloprid @ 50 PPM + Spinosad @ 1000 PPM + Silymarin @ 1000 PPM in feed

All birds have free access to fresh feed and water *ad libitum* throughout the experimental period.

Growth Rate

Individual body weights of all the birds were recorded by using electronic balance on day one and subsequently on 7th, 14th, 21st and 28th day of experiment to study the body weight gains.

Histopathology

The tissue samples of liver (1×1 cm3) were collected and fixed in 10% neutral buffer formalin (NBF) soon after sacrifice. The samples were processed, sectioned (5µm) and stained with Hematoxylin and Eosin (H&E) for histopathological examination as per the standard procedure (Luna, 1968) [10].

Statistical Analysis

Data obtained were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16.0. Differences between means were tested by using Duncan's multiple comparison tests and significance level was set at $P < 0.05$ (Snedecor and Cochran, 1994) [14].

Results and Discussion

Body weight

There is a significant reduction in body weight in group 2, 3 and 4 when compared to control This decrease in body weight gain is due to decreased feed and water intake as a result of hepato, renal toxicity. The findings in group 2 are in accordance with the earlier reports of Koshlukova (2006) and Sasidhar babu *et al.* (2014) [9, 13] and the findings in group 3 were in agreement with Yano *et al.* (2002) Mansour *et al.* (2007) [11]. In amelioration groups i.e. group 5 and 6 showed a significant improvement in comparison with group 4 indicating the protective action of ameliorating agents.

Table 2: Weekly body weight gain (g) in different groups.

Group	Day 7	Day 14	Day 21	Day 28
Group 1	110.88±1.28^{a}	156.6±8.72^{a}	367.68±16.17^{a}	366.28±31.55^{a}
Group 2	100.9±1.96^{b}	126.27±8.33^{b}	312.61±17.64^{b}	264.34±6.85^{b}
Group 3	100.05±2.19^{b}	125.15±2.85^{b}	297.66±7.37^{b}	264.11±2.96^{b}
Group 4	87.53±4.78^{c}	100.6±10.54^{c}	202.62±18.91^{c}	204.86±4.04^{c}
Group 5	99.03±1.02^{b}	123.72±3.49^{b}	256.55±25.66^{b}	276.70±28.53^{b}
Group 6	97.55±2.79^{b}	123±5.25^{b}	264.76±14.99^{b}	273.51±15.38^{b}
P value	*	*	*	*

Values are Mean ± SE (n=6); one way ANOVA

Means with different superscripts in a column differ slightly at P<0.05 (*).

4.6 Histopathology

4.6.1 Liver

Histologically, significant lesions like marked dilation and congestion of central vein, degeneration of hepatocytes and also sinusoidal dilation in group 2 were in agreement with the observations of Sasidhar Babu *et al.* (2014) [13] in layer birds, Omiama (2004) [12] in male Japanese quails, Kammon *et al.* (2010) [8] in layer chicken and Soujanya *et al.* (2013) [15] in male rats. Histologically the lesions found in 3rd group liver sections were moderate to severe dilation of central vein, severe dilation of sinusoids, varied degrees of degeneration in hepatocytes and focal infiltration of mononuclear cells indicating hepatotoxicity induced by SPD. Similar observations were recorded in male rats by Aboul-Enein *et al.* (2012) on oral administration of SPD @ 347.49 mg/Kg b. wt. Section of 4th group liver revealed severe congestion and dilation of central vein and sinusoidal spaces, necrosis of liver and focal area of lymphocytic infiltration. The severity of changes might be due to cumulative accumulation of metabolites of IM and SPD and its combination. The liver is the principal target organ for detoxification of any intoxicants. In the course of degenerative changes, repair and regeneration few cells might undergo the process of necrosis due to covalent binding of reactive electrophilic metabolites to liver macromolecules (Gardner and Cluff, 1970) [4]. The microscopic changes in group 5 liver are moderate dilation of

central vein and sinusoidal spaces. In group 6, the liver sections revealed a moderate to severe vacuolar degeneration, severe dilation of central vein and sinusoids. On perusal of literature no work has been carried out on mixed toxicity by IM+SPD and its amelioration. The vacuolation of hepatocytes might be due to retention of fluid inside the cell resulting in cloudy swelling which might be due to reduction of energy necessary for regulation of ion concentration of the cells/hypoxia/oxidative stress (Omiama, 2004) [12].

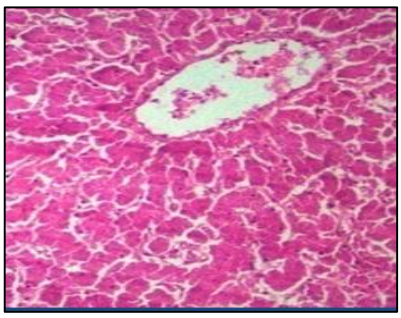

Fig 1: Photomicrograph of liver showing dilation and mild congestion of central vein with dilated sinusoidal spaces (Group 2, day 28): H&E 50 µm

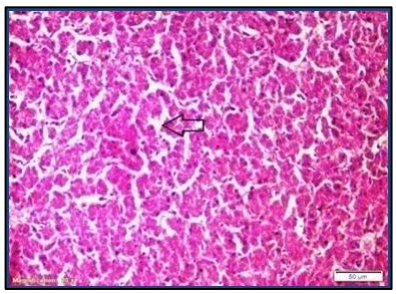

Fig 2: Photomicrograph of liver showing degeneration of hepatocytes and severe dilation of sinusoidal spaces (arrow) (Group 3, day 28): H&E 50 µm

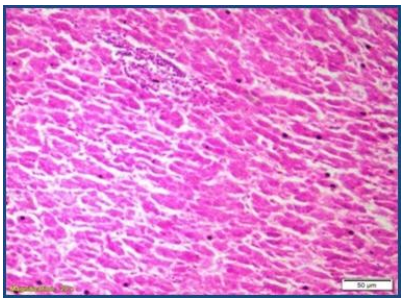

Fig 3: Photomicrograph of liver showing necrosis of hepatocytes and focal area of round cell infiltration (Group 4, day 28): H&E 50 µm

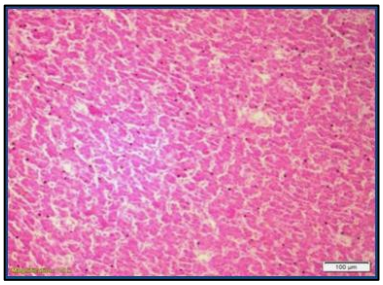

Fig 4: Photomicrograph of liver showing severe dilation of sinusoidal spaces (Group 4, day 28): H&E 100 µm

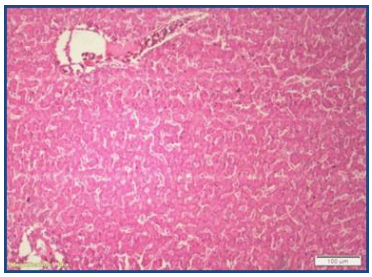

Fig 5: Photomicrograph of liver showing mild to moderate congestion in central vein (Group 5, day 28): H&E 100 µm

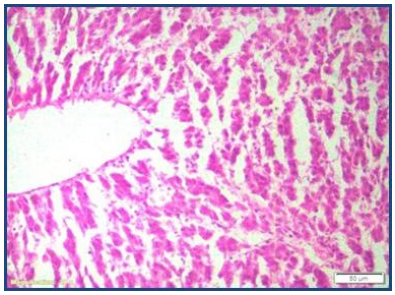

Fig 6: liver showing severe dilation of central vein, degenerating hepatocytes and sinusoidal spaces (Group 6, day 28): H&E 50

4.6.2 Kidney

Histologically, kidney section of group 2 revealed shrunken glomeruli and increased Bowman‟s space (Fig.7). A note worthy changes like cystic dilation and degeneration of tubules was observed which were in accordance with Kammon *et al.* (2010) [8] in layer chickens and Soujanya *et al.* (2013) [15] in male rats. Kidneys are major excretory organs for many xenobiotics and intoxicants indicated the renal damage caused by IM (Soujanya *et al.*, 2013) [15]. Group 3 kidneys have shown increased interstitial space, moderate to severe degeneration and necrosis of tubular epithelial cells. These findings were in agreement with observation of Hanley *et al.* (2002) [2] in mice. Group 4 kidneys revealed a mild cystic dilation, intertubular haemorrhage and degeneration of tubules. Mild increase in bowman space and focal to diffuse lymphocytic infiltration was noticed in group 5 birds. Group 6 kidney section revealed mild hypercellularity at areas of degenerated glomeruli and moderate to marked dilation of tubules. On perusal of literature no work has been carried out on mixed toxicity by IM+SPD and its amelioration. The changes were due to nephrotoxic effects of IM and SPD and its combination, amelioration of mixed toxicity with VE (group 5) and SIL (group 6) revealed insignificant changes indicating that VE and SIL incorporation in feed might be initiated the process of repair and regeneration caused by IM and SPD.

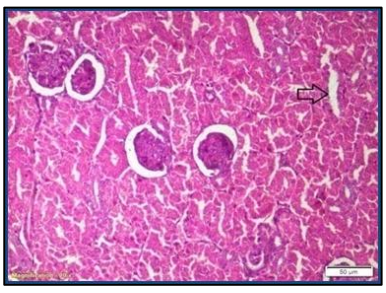

Fig 7: Photomicrograph of kidney showing cystic dilation of tubules (arrow) and increased bowman space (Group 2, day 28): H&E 50 µm

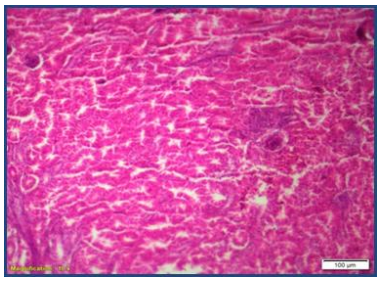

Fig 8: Photomicrograph of kidney showing moderate to severe degeneration of tubular epithelial cells (Group 3, day 28): H&E 100 µm

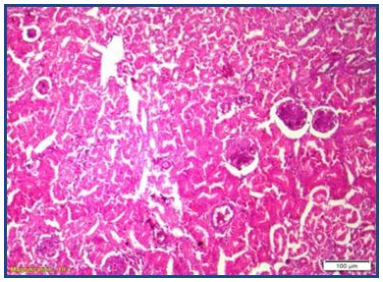

Fig.9: Photomicrograph of kidney showing degeneration of tubular epithelial cells and increase in bowman space (Group 4, day 28): H&E 100 µm

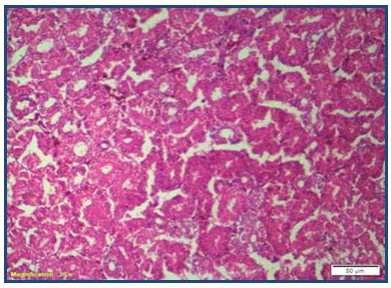

Fig 10: Photomicrograph of kidney showing mild cystic dilation of tubular epithelial cells (Group 4, day 28): H&E 50 µm

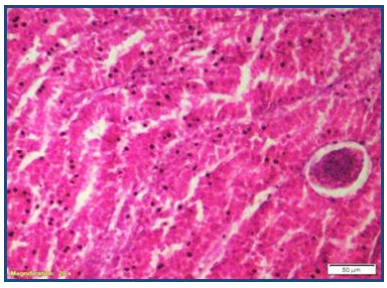

Fig 11: Photomicrograph of kidney showing mild to diffused round cell infiltration and mild increase in bowman space (Group 5, day 28): H&E 50 µm

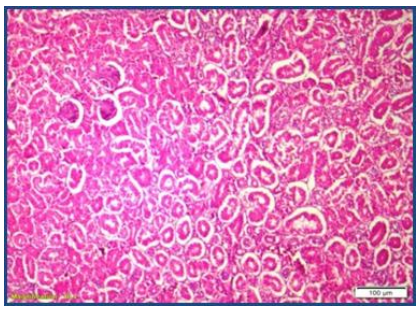

Fig 12: Photomicrograph of kidney showing moderate to marked dilation of tubules (Group 6, day 28): H&E 100 µm

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