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Effect of Spinosad and Imidacloprid on Serum Biochemical Alterations in Male Broilers and Its Amelioration with Vitamin E and Silymarin

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A B S T R A C T

The present experiment was conducted on 120 male broiler chicks which were divided into six groups of 20 each. Group 1 served as control, group 2 treated with imidacloprid @ 50 ppm in feed, group 3 was treated with spinosad @ 1000 ppm in feed, group 4 was treated with imidacloprid @ 50 ppm and spinosad @ 1000 ppm in feed, group 5 was treated with imidacloprid @ 50 ppm, spinosad @ 1000 ppm and Vitamin E @ 20 ppm in feed and group 6 was treated with imidacloprid @ 50 ppm, spinosad @ 1000 ppm and silymarin @ 1000 ppm in feed. The experiment was carried out for 4 weeks. The blood samples were collected for separation of serum to analyze various biochemical parameters on 14 and 28 days after oral administration the compounds mentioned above. The biochemical assays showed a significant ($P < 0.05$) increase in serum ALP, significant ($P < 0.05$) decrease in serum total protein, insignificant decrease in serum AChE and an insignificant increase in serum ACP in all groups when compared to control. Group 5 and 6 showed numerically decreased values of serum ALT and increase in TP when compared to group 4. Liver tissue biochemical profile revealed a significant ($P < 0.05$) reduction in GSH concentration in liver of group 2, 3 and 4 and a mild to moderate improvement was noticed in group 5 and 6 when compared to group 4. These results revealed that exposure of Imidacloprid, spinosad and its combination resulted in alterations in serum and tissue biochemical parameters.

Introduction

In the resume of success of green revolution the usage of insecticides and pesticides has been enormously increased in grain crop cultivation. However, their indiscriminate use lead to widespread concern because of their potential adverse effect on animal and human health (Al-saleh, 1994). 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

(IM) is a potent and most widely used insecticide introduced in the market in 1991 (Yamamoto and Casida, 1999). The mode of action of spinosad (SPD) is *via* a neural mechanism (Orr *et al.,* 2009). SPD primarily targets binding sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system that are distinct from those at which other insecticides have their activity. IM acts as an agonist on post-synaptic nicotinic acetylcholine receptor (nAChR) in insects (Tomizawa and Yamamoto, 1993 and Tomizawa *et al.,* 2005). The selective toxicity of IM is due to its high affinity for insect nAChR when compared to mammals (Liu and Casida, 1993; Chao and Casida, 1997 and Zhang *et al.,* 2000). This binding process is irreversible (Ware *et al.,* 2004).

Both vitamin E (VE) and Silymarin (SIL) have an antioxidant effects independently and when given together may enhance the immunoprotective and immunostimulatory properties of each other (Horvath *et al.,* 2001). In view of the significant adverse effects induced by SPD and IM, the current study was designed to evaluate the mixed toxicity and its pathological effect in broiler chicken and to study the ameliorative effect of VE and SIL to overcome the mixed toxic effects.

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 (Venkateswara Hatcheries Pvt. Ltd. Hyderabad) and were vaccinated against Marek's Disease (MD) at the hatchery itself. On arrival, the chicks were individually weighed, wing banded and divided into six groups of 20 each. 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.

Collection of blood for serum separation

Approximately 3 mL of blood was collected from each bird (from wing vein) into a clot promoting [(Vit K- coated-clot activator tube-plain 13mm x 75mm, 5mL) (Rapid Diagnostics Pvt. Ltd., Delhi)] vaccutainers and allowed to clot for 3-4 hours, later centrifuged (Sigma 1-13-bench top laboratory centrifuge, USA) at 20k rpm for 10 minutes, serum was separated into Eppendorf tubes and stored at -20^0C, subsequently used for serum biochemistry [Alkaline phosphatase (ALP) as per modified international federation of clinical chemistry (IFCC) methods, serum total protein as per the standard Biuret procedure, Acid phosphatase as per α Naphthylphosphate Kinetic method and Cholinesterase as per butyryl thio choline method] by using semi-automatic biochemical analyzer. All the values were tabulated for statistical analysis.

Reduced glutathione (GSH) (Moron *et al.,* 1979)

The method is based on reaction of reduced glutathione (GSH) with 5-5[1] dithiobis-2-nitrobenzoic acid (DTNB) to give a compound that absorbs light at 412 nm.

Results and Discussion

The present experiment was designed to study the mixed toxicity of SPD and IM in broilers and its amelioration with VE and SIL at definite time periods in different groups.

Biochemical parameters

Total protein concentration (g/dL)

Significantly ($P < 0.05$) decreased mean values (4.16 and 4.06) of total protein were observed in group 4 and elevated mean value (5.22 and 5.43) was recorded in group 1

during 14th and 28th day of the experiment. Compared with group 4, there was an insignificant increase in total protein recorded on 14th day and at the end of experiment in group 2, 3, 5 and 6 whereas the values were significantly ($P < 0.05$) lower than group 1 (Table 2). The total protein levels were significantly ($P< 0.05$) reduced in 2, 3, 4, 5 and 6 group birds when compared to that of control. The observations in group 2 were similar to the results of Siddiqui *et al.,* (2007) and Sasidhar Babu *et al.,* (2014) in birds for a period of 28 and 90 days respectively. The observations in group 3 were in accordance with the results of Stebbins *et al.*, (2002) in experimental mice for a period of 13 weeks.

On perusal of literature, no recorded evidences were available about mixed toxicity due to IM+SPD and its amelioration with VE and SIL. Liver is the major site for protein synthesis. The significant ($P< 0.05$) reduction in total protein in the present experiment might be due to oxidative stress and induced hepatotoxicity. The other reason for decrease in total serum protein might be due to less feed and water intake in general. There was an insignificant increase in serum total protein in group 5 and 6, but significantly lower when compared to group 4 and 1 respectively. This difference might be due to antioxidant property of VE and SIL in group 5 and 6 respectively.

Serum acetyl cholinesterase enzyme (U/L)

There was an insignificant reduction in serum acetyl cholinesterase on 14th and 28th day even though decreased mean value (1459.40±182.52) was observed in group 4 and highest mean value (1865.60±525.91) was observed in group 1 on day 14. On 28th day the lowest mean value (1322.90±128.81) was observed in group 4 and the highest mean value (1669.80±191.52) was recorded in group 1 (Table 2). An insignificant decrease in serum acetyl cholinesterase was noticed in 2, 3, 4, 5 and 6 group birds when compared to that of control. A significant ($P < 0.05$) decrease in acetyl cholinesterase activity was reported by Aboul-Enein *et al.,* (2012) in male rats administered with 347.49 mg/Kg b. wt of SPD for 4 weeks and Kammon *et al.,* (2010) recorded an insignificant change in plasma acetyl cholinesterase levels in imidacloprid intoxicated chickens.

Both toxins will act by excitation of the nervous system with activation of nicotinic acetylcholine receptors (nAChRs), SPD acts by inhibition of the AChE whereas IM acts by binding with acetylcholine both actions resulting in hyperexcitation.

In group 5 and 6 there was an insignificant increase in AChE when compared to group 4 which indicates the protective action of VE and SIL respectively.

Serum acid phosphatase (U/L)

There was an insignificant increase in serum acid phosphatase on 14th and 28th day of the experiment. There was no significant ($P < 0.05$) increase in serum acid phosphatase in 2, 3, 4, 5 and 6 group birds when compared to that of control on 14th and 28th day of experiment (Table 3). Acid phosphatase is an enzyme stored in lysosomes of animal and plant species which is responsible for bone resorption in animals. The insignificant increase indicates that bone resorption does not occur due to mixed toxic effects though the bone related parameters were not included in this experiment.

Serum alkaline phosphatase (IU/L)

A significant increase ($P < 0.05$) in serum alkaline phosphatase (ALP) was observed in 2, 3, 4, 5 and 6th group birds when compared with group 1 birds (Table 3).

Table.1 Experimental design

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

Table.2 Total protein (gm/dL) and serum acetylcholinesterase enzyme (U/L) in different groups

	Total protein (gm/dL)		Serum Acetylcholinesterase enzyme (U/L)	
Group	**Day 14**	**Day 28**	**Day 14**	**Day 28**
Group 1	5.22±0.20^{a}	5.43±0.28^{a}	1865.60±525.91	1669.80±191.52
Group 2	4.55±0.28^{b}	4.70±0.15^{b}	1816.70±135.65	1656.20±207.35
Group 3	4.42±0.16^{b}	4.68±0.14^{b}	1746.90±123.00	1561.50±159.55
Group 4	4.16±0.11^{b}	4.06±0.15^{b}	1459.40±182.52	1322.90±128.81
Group 5	4.60±0.16^{b}	4.64±0.26^{b}	1606.20±352.37	1553.10±189.89
Group 6	4.42±0.11^{b}	4.50±0.17^{b}	1554.20±195.28	1484.40±234.96
P value	*	*	NS	NS

Values are Mean ± SE (n=6); one way ANOVA
Means with different superscripts in a column differ slightly at P<0.05 (*).
NS: Non significant

Table.3 Serum acid phosphatase (U/L) and serum alkaline phosphatase (U/L) in different groups

	Serum Acid Phosphatase (U/L)		Serum Alkaline Phosphatase (U/L)	
Group	**Day 14**	**Day 28**	**Day 14**	**Day 28**
Group 1	2.20±0.50	1.96±0.39	105.04±6.60^{c}	104.64±5.15^{b}
Group 2	2.67±0.76	2.01±0.12	119.39±2.46^{b}	119.63±2.92^{a}
Group 3	3.38±0.85	2.37±0.23	120.18±3.00^{ab}	121.47±3.47^{a}
Group 4	3.54±0.96	2.99±0.61	133.17±2.47^{a}	134.95±2.31^{a}
Group 5	3.06±0.91	2.31±0.40	125.68±3.62^{ab}	125.28±3.77^{a}
Group 6	3.10±0.43	2.56±0.97	126.85±5.08^{ab}	128.55±9.22^{a}
P value	NS	NS	*	*

Values are Mean ± SE (n=6); one way ANOVA
Means with different superscripts in a column differ slightly at P<0.05 (*).
NS: Non significant

Table.4 Reduced glutathione (GSH) concentration in liver (µM/mg) in different groups

Group	Day 14	Day 28
Group 1	192.02±2.45[a]	191.72±2.28[a]
Group 2	161.42±2.03[b]	163.55±9.86[b]
Group 3	161.79±9.18[b]	164.22±13.13[b]
Group 4	129.38±9.90[c]	129.72±8.45[c]
Group 5	146.12±9.21[bc]	140.7±6.38[bc]
Group 6	142.09±17.31[bc]	139.27±10.29[bc]
P value	*	*

Values are Mean ± SE (n=6); one way ANOVA
Means with different superscripts in a column differ slightly at P<0.05 (*).

The observations in group 2 were in agreement with experimental results of in Kammon *et al.,* (2010) and Mohany *et al.,* (2012) in layer chicken and rats respectively in IM induced studies. Increased levels of ALP in group 3 were in agreement with Stebbins *et al.,* (2002) and Yano *et al.,* (2002). An elevated level of serum ALP in the present study might be due to toxicity of IM, SPD and its combination which triggered the process of inflammation in liver, kidney and other organs which were evidenced histopathologically.

In group 5 and 6 there was an insignificant decrease in mean value of ALP when compared with group 4 indicating the initiation of mild protective action of VE and SIL respectively, which was supported histologically.

On perusal of literature, no recorded evidences were available about mixed toxicity and also their amelioration with VE and SIL.

Tissue biochemical profile

Reduced glutathione (µM/mg)

Reduced glutathione (GSH) concentration was one of the most essential non-enzymatic antioxidants for detoxification of several exogenous and endogenous intoxicants in liver. It has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione (GS-SG) and other disulphides (Umalaksmi and Devaki, 1992). It acts as an essential cofactor for antioxidant enzymes including glutathione peroxidase (GPx) and Glutathione S-transferase (GST) (Hayes *et al.,* 2005). Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify peroxides produced due to increased lipid peroxidation (Cathcart, 1985). The depletion of GSH predisposes the cells to oxidative damage (Khan *et al.,* 2005).

In the present study, a significant ($P < 0.05$) reduction in GSH concentration (hepatic tissue) was observed in group 2, 3 and 4. Contrary to this, in group 5 and 6 there was an insignificant increase in GSH concentration, this might be due to an initiation of hepatoprotective action of VE and SIL respectively (Table 4).

Similar observations were recorded in IM induced experimental studies by Sasidhar Babu *et al.,* (2014) in layer birds with the combination of herbal and Vit. C as ameliorating agents and Soujanya *et al.,* (2013) conducted an experiment in male rats with Vit. C as an ameliorative agent. The findings in group 3 were in accordance with

Aboul-Enein *et al.,* (2012) in rats. On perusal of literature, no recorded evidences were available about mixed toxicity due to IM+SPD and also their amelioration with VE and SIL. The reduced glutathione signifies the generation of free radicals following mixed intoxication (IM+SPD) resulting in exhaustion of the GSH during oxidative stress. This depletion in GSH level suggested that there was an increased peroxidation.

Based on the results obtained in the present study, it can be concluded as that IM, SPD and its combination resulted in mild, marked and severe biochemical alterations at different time intervals. The present dose levels (IM @ 50 ppm and SPD @ 1000 ppm) were found to be hepatorenal toxic in nature. A change in tissue oxidative parameter (liver GSH concentration) was indicating that IM, SPD and its combination were responsible to cause oxidative stress. The co-administration of vitamin E and silymarin @ 20 and 1000 ppm revealed mild improvement in all the above parameters as a part of initiation in repair and regeneration.

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