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Kitazin, an Organophosphorus Pesticide Induced Pathomorphological Alterations in Broiler Chicken

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ABSTRACT

A research was conducted in broiler chicken to study the pathological effects of an organophosphorous fungicide, Kitazin used against rice blast in paddy. To assess the acute toxic effect, 48 day old broiler chicks were randomly divided in four groups of 12 birds each. After one week of acclimatization period, a single oral dose of Kitazin was administered @ 300 mg, 600 mg and 900 mg/ Kg body weight to group 2 (T_1), group 3 (T_2) and group 4 (T_3) respectively. To assess the sub acute toxic effect, 48 day old broiler chicks were randomly divided in four groups of 12 birds each. After one week of acclimatization period, Kitazin was incorporated in the feed @ 100 ppm, 200 ppm and 400 ppm to group 2 (T_1), group 3 (T_2) and group 4 (T_3) respectively for a period of 6 weeks. In both the studies the group 1 birds served as control (C). A detailed necropsy examination of different experimental groups was conducted on 14th and 21st day of age in acute study and on 49th day for sub acute study. Grossly, severe congestion of most of the visceral organs and reduction in the size of lymphoid organs in both acute and subacute toxicity with Kitazin were observed. Histopathologically, there were degeneration of hepatocytes, mononuclear infiltration, nephrosis and necrosis in visceral organs. Brain showed perivascular cuffing, chromatolysis, neuronophagia and spongiform changes. From the present study the immunosuppressive and pathological effects of kitazin were confirmed and the effects found to be dose dependent.

HIGHLIGHTS

- Kitazin has adverse effects on general health, body weight and immune status of the birds.
- Kitazin induced effects are dependent on dose and duration of its exposure. Histopathological findings confirm the immunosuppressive properties of the chemical even with low levels of exposure.

Keywords: Kitazin, Organophosphorous, Broiler, Pathological, Lymphoid, Nervous tissue

Industrial agriculture is the predominant form of agriculture being practiced worldwide and relies heavily on the chemical pesticides for control of crop diseases. Kitazin (also known as Iprobenfos, IBP) is an organophosphorus pesticide used against rice blast caused by *Pyricularia oryzae*. It is a systemic fungicide of phosphorothioate group, chemically known as S- Benzyl O, O-diisopropyl phosphorothioate. It inhibits the biosynthesis of phosphatidyl choline, an important component of the fungal cell membrane. Several organophosphous pesticides

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found to induce pathological changes in vital organs of different poultry species. Wani *et al.* (2018) studied the chlorpyrifos induced pathomorphological alterations in liver, kidney and brain of broilers and reported that, the compound caused degeneration and necrosis in liver, kidney tubules and engorgement of cerebral blood vessels and inflammation. Mishra and Srivastava, (2015) reported that organophosphate pesticides are the most toxic synthetic chemicals that induce acute and chronic poisoning. The organophosphates including Iprobenfos found to reduce the antioxidant levels in liver and brain tissues of rats and thereby causing tissue damage. The studies on toxicological effects of iprobenfos in poultry are limited. Keeping in view of the above, the present study was planned to assess the pathological effects of Kitazin in broiler chicken.

MATERIALS AND METHODS

For this experiment, ninety six number of day old commercial broiler chicks (VENCOBB strain) were procured from Venkey's (India) Limited. Among these, 48 chicks were used to study the acute toxic effect and another 48 were used to study the sub acute toxic effect. The chicks were vaccinated with Ranikhet disease vaccine F1 strain on 5th day of experiment. The chicks meant for sub acute study were given booster dose of the same vaccine on 30th day.

The experiment was conducted in two phases; the acute and sub acute study. In the acute study, after one week of acclimatization period, a total of 48 chicks were randomly divided in four groups of 12 birds each, and a single dose of Kitazin was administered orally @ 300 mg/Kg body weight (T_1), 600 mg/Kg body weight (T_2) and 900 mg/Kg body weight (T_3) to group 2, 3 and 4 respectively. In the sub acute study, after one week of acclimatization period, a total of 48 chicks were randomly divided in four groups of 12 birds each, and Kitazin was incorporated in the feed @ 100 ppm (T_1), 200 ppm (T_2) and 400 ppm (T_3) to group 2, 3 and 4 respectively for a period of 6 weeks. In both the studies, the group 1 birds served as control (C). The chicks were fed commercial broiler mash and *Ad libitum* supply of fresh water and feed were provided throughout the experimental period. Kitazin 48% E.C. was procured and used for the study.

A detailed necropsy examination of all the birds of different experimental groups was performed on 14th and 21st day of age in acute toxicity study and on 49th day in sub acute toxicity study. The birds that died during the course of experiment, necropsy was done immediately and the gross lesions were studied. Weight of the organs like liver, heart, lungs bursa, kidney, spleen, brain and pancreas were measured and presented as organ weight/ body weight ratio. The tissue pieces from liver, kidney, lungs, spleen, bursa of Fabricius, intestine and brain were collected in 10% buffered formalin solution for histopathological examination. Formalin fixed tissues were processed and stained with hematoxylin and eosin following the procedure of Culling (2014). The data obtained in the present study were analyzed statistically and subjected to test of significance as per the methods described by Snedecor and Cochran (1994). A value of $P < 0.05$ was considered as statistically significant.

RESULTS

Organ to body weight ratio

In the acute study, on 21st day of age, a highly significant decrease in weight of liver in T_2 (2.87 ± 0.02) and T_3 groups (2.84 ± 0.02) and a significant decrease ($P<0.05$) in weight of liver in T_1 group (3.19±0.03) was noticed as compared to control group (3.34±0.01). The decrease in weight of spleen was highly significant ($P<0.01$) in T_3 group (0.09±0.01) and significant ($P<0.05$) in T_1 (0.10±0.01) and T_2 groups (0.10±0.00) as compared to the control (0.13±0.00). A significant decrease ($P<0.05$) in weight of bursa was also found in T_3 group (0.25±0.04) in comparison to control (0.32±0.00). The weight of other organs like heart, lungs, kidney, brain and pancreas also showed a non significant decrease ($P>0.05$) in weight in the treatment groups.

In sub acute study, at the end of experiment (on 49th day of age), the weight of liver in control, T_1, T_2 and T_3 groups were 3.40±0.03, 3.28±0.05, 3.18±0.08 and 3.07±0.04 respectively, in which the decrease in T_2 was significant ($P<0.05$) and that of T_3 was highly significant ($P<0.01$) in comparison to control. A significant decrease ($P<0.05$) in weight of heart, bursa, spleen and pancreas was also observed in T_3 group. The T_1 and T_2 groups showed a non significant decrease ($P>0.05$) in weight of these organs in comparison to the control.

Gross pathology

Acute

The post-mortem examination of the birds that died during the course of experiment in T_2 and T_3 groups revealed lesions of severe congestion of visceral organs including liver, kidney and brain. Congestion of lungs and upper respiratory tract was also observed. The intestines were fluid filled and revealed congestion.

Necropsy examination of T_2 and T_3 group birds on 14th and 21st day of age, showed congestion of liver and lungs. Congestion of trachea, kidney, brain and intestines were also observed. Atrophied spleen and bursa were also noticed in T_2 and T_3 groups and the severity was more pronounced in T_3 group than in T_2 group. The birds from T_1 group also showed congestion of liver, kidney and lungs. The birds from control group did not show any observable gross lesions in any of the organs throughout the experimental period.

Sub acute

In sub acute study, severe congestion of liver and oedema, emphysema and congestion of lungs were observed in T_2 and T_3 groups. Focal areas of necrosis were also observed in liver and spleen. Atrophy of spleen and bursa were noticed in comparison to the control. Other organs like intestines, kidneys and brain also showed congestion. Birds from T_1 and T_2 groups showed congestion of liver, kidneys, lungs and intestine. No observable gross lesions were detected in the brain of T_2 and T_1 group birds.

Histopathology

The tissue samples of liver, kidneys, lungs, brain, spleen, bursa and intestine from the routine mortality and sacrificed birds were collected and fixed in 10 % buffered formalin solution for histopathological examination.

Liver

Acute: On 14th day, liver from T_3 group birds had lesions of hyperemia, vacuolar degeneration of hepatocytes, periportal mononuclear cell infiltration particularly by lymphocytes and macrophages, focal areas of necrosis (3-4 mm size) and dilatation of sinusoids. Birds from T_2 and T_1 groups also showed periportal hepatitis and focal areas of necrosis. On 21st day of age, the changes observed were hyperaemia, mononuclear cell infiltration, dilatation of sinusoids, degeneration and necrosis in all the treatment groups and the severity was more pronounced in the higher dosage group.

Sub acute: The liver from T_3 group birds showed hyperaemia, dilatation of sinusoids, periportal hepatitis with infiltration of macrophages and lymphocytes and the hepatocytes were in various stages of degeneration (Fig. 1&2). Birds from T_2 group showed areas of extensive necrosis, diffuse infiltration of mononuclear cells and severe vacuolar degeneration. Birds from T_1 group also showed hyperaemia, diffuse hepatitis with mononuclear cell infiltration and focal areas of necrosis. Liver from the control group did not show any significant histopathological changes**.**

Kidney

Acute: The lesions on 14th and 21st day of age were of degeneration and necrosis, haemorrhage, presence of coagulated protoplasm (albumin) in glomeruli and nephrosis in T_2 and T_3 group birds. Birds from T_1 group also showed degenerative changes.

Subacute: The birds from T_3 group showed various stages of degeneration along with accumulation of coagulated protoplasm within the glomeruli and nephrosis (Fig. 3&4). The lesions from the T_2 group birds include diffuse haemorrhage, hyperaemia, glomerular nephritis and nephrosis. Hyaline glomerular nephrosis was also observed. Degenerative changes were observed in the T_1 group birds.

Lungs

Acute: On 14th day of age, lungs from T_3 group birds showed haemorrhage, oedema, emphysema, infiltration of inflammatory cells and desquamation of bronchial epithelium. Similar lesions were observed in all the treatment groups on 21st day of age also.

Sub acute: Lungs from T_3 group birds showed severe congestion, oedema, emphysema and extensive haemorrhages throughout. Desquamation of bronchial

epithelium and infiltration of inflammatory cells were also observed. Birds from T_1 and T_2 groups showed haemorrhage, oedema and emphysema. In both the studies, birds from control group did not show any changes (Fig. 5).

Brain

Acute: The changes in the brain of birds that died from T_3 and T_2 groups during the course of experiment were congestion, spongiform changes, neuronal degeneration, perivascular cuffing, chromatolysis and neuronophagia. The lesions on 14th and 21st day of age were severe meningitis, spongiform changes, perivascular cuffing, chromatolysis and neuronophagia. Degeneration of Purkinje cells of cerebellum was also observed (Fig. 6).

Sub acute: The birds from T_3 group showed severe spongiform changes, meningitis, satellitosis, chromatolysis and neuronophagia. The same lesions were also observed from T_2 and T_1 group birds along with perivascular cuffing. The cerebellum of T_3 group birds showed degenerated Purkinje cells (Fig. 7).

In general, the extent of spongiform changes and neuronophagia were more pronounced in birds treated with high dose levels of Kitazin (T_3 and T_2 groups) in comparison to birds administered low levels of Kitazin (T_1) in both acute and sub acute studies.

Spleen

Acute: The spleen showed diffuse necrosis and haemorrhages in all the treatment groups both on 14th and 21st day of age. Depletion of lymphoid tissue was observed in all the treatment groups whereas the lymphoid tissue was found intact in the control birds.

Sub acute: The birds showed extensive degeneration and necrosis in all the treatment groups (Fig. 8).

Bursa

Acute: The bursa showed degenerative and necrotic changes in lymphocytes leading to depletion of lymphoid tissue in Kitazin fed birds.

Sub acute: In sub acute study also similar lesions were observed. In control birds the lymphoid tissue of bursa was found intact.

Intestines

Acute: The changes in the intestines were, disruption of villi and enteritis characterised by infiltration of inflammatory cells.

Sub acute: The intestine of birds from T_3 group showed lesions of villi disruption and chronic suppurative enteritis with infiltration of lymphocytes and proliferation of fibroblasts.

DISCUSSION

Organ/ Body weight ratio

In acute toxicity with Kitazin, a significant reduction in the weight of liver, spleen and bursa were noticed. Sub acute toxicity resulted in significant reduction in the weight of liver, heart, spleen, bursa and pancreas. Garg *et al*. (2004) reported a reduction in the weight of spleen, thymus and bursa of Fabricius in broiler chicken treated with monocrotophos. Microscopic examination of these organs further revealed atrophy/ hypoplasia, decrease in size of the follicles with depletion of lymphocytes in the lymphoid follicles and haemorrhages in the thymus. The reduction in size of lymphoid organs gives an indication of their structural alteration as well as reduced functional activity. The significant reduction in organ weight recorded in pesticide intoxication may be due to reduction in the parenchymatous or functional cells (Garg *et al*., 2004).

Gross and histopathology

The gross lesions observed in the present study were severe congestion of visceral organs including liver, kidney, lungs, trachea, intestine and brain in acute toxicity of Kitazin. Oedema of lungs, fluid filled intestines and reduction in the size of spleen and bursa were also noticed. In the sub acute toxicity, congestion of lungs, liver, kidneys, intestines and brain were noticed. Focal areas of necrosis in liver and spleen and reduction in the size of spleen and bursa were also observed.

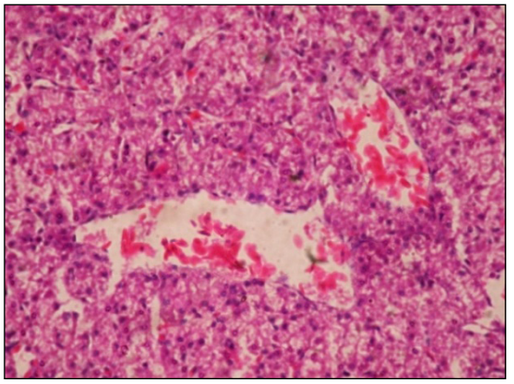

Fig. 1: Photomicrograph of liver showing hyperaemia and hepatocytes undergoing various stages of degeneration and necrosis with Kitazin @ 400 ppm on 49th day (H&E, 400X)

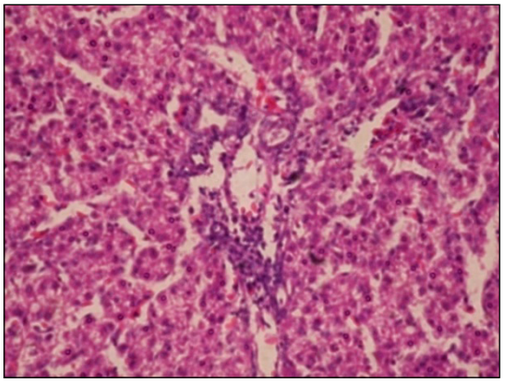

Fig. 2: Photomicrograph of liver showing periportal hepatitis with infiltration of inflammatory cells and sinusoidal dilatation with Kitazin @ 400 ppm on 49th day (H&E, 400X)

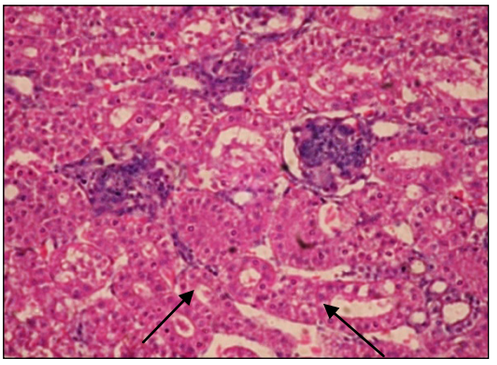

Fig. 3: Photomicrograph of kidney showing glomerular nephritis, and solid, dense appearing masses of coagulated protoplasm in tubules (indicated by arrows) with Kitazin @ 400 ppm on 49th day (H&E, 400X)

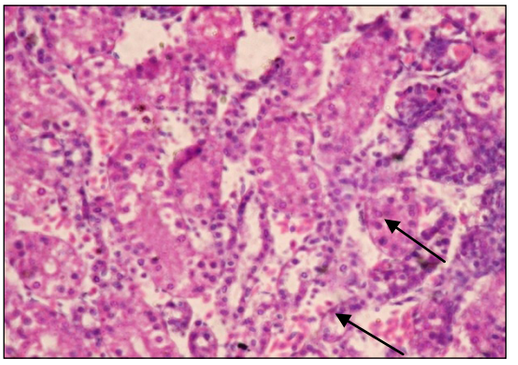

Fig. 4: Photomicrograph of kidney showing tubular nephrosis and solid, dense appearing masses of coagulated protoplasm in tubules (indicated by arrows) with Kitazin @ 400 ppm on 49th day (H&E, 400X)

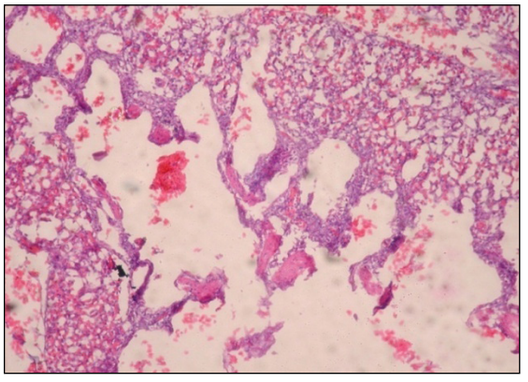

Fig. 5: Photomicrograph of lungs showing congestion, haemorrhage, emphysema and atelectasis with Kitazin @ 200 ppm on 49th day (H&E, 200X)

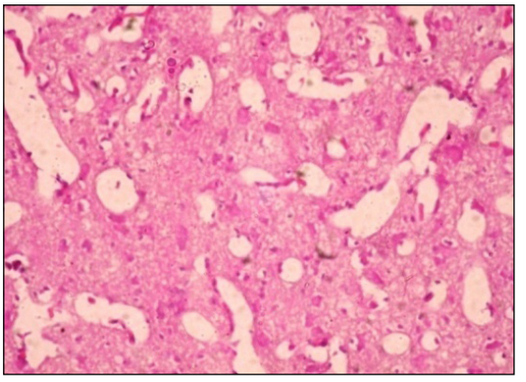

Fig. 6: Photomicrograph of brain showing neuronophagia and extensive spongiform changes with Kitazin @ 900 mg/Kg body weight on 14th day (H&E, 400X)

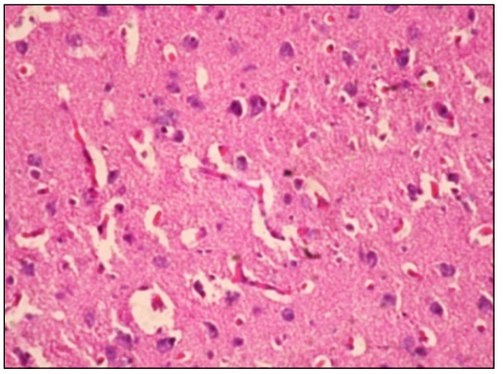

Fig. 7: Photomicrograph of brain showing chromatolysis, neuronophagia and spongiform changes with Kitazin @ 400 ppm on 49th day (H&E, 400X)

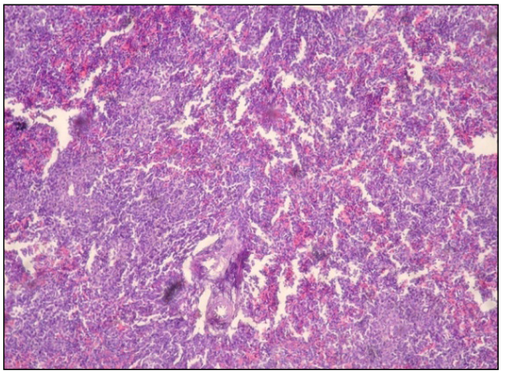

Fig. 8: Photomicrograph of spleen showing haemorrhage and degenerative changes with Kitazin @ 200 ppm on 49th day (H&E, 200X)

Histopathological examination of liver showed changes such as hepatocytes at various stages of degeneration, focal areas of necrosis, and the infiltration of lymphocytes and macrophages around the necrotic areas. Hyperemia, vacuolar degeneration and periportal mononuclear cell infiltration were also reported. Severe nephrosis in kidney, degenerative changes and necrosis in spleen and bursa and disruption of intestinal villi were also observed. Lungs revealed desquamated bronchial epithelium, congestion, oedema, atelectasis and emphysema. The lesions in the brain were meningitis, congestion, neuronal degeneration, perivascular cuffing, chromatolysis, neuronophagia and spongiform changes. Similar lesions were also reported by Singh (2003) who observed the changes such as vacuolar degeneration, necrosis and mononuclear cell infiltration in liver; and degenerative and necrotic changes along with rarefaction of lymphoid cells in spleen, bursa of Fabricius and thymus with chlorpyriphos toxicity in poultry. Khaji (2007) also observed the dilatation of sinusoids and vacuolar degeneration of liver; degeneration of tubular epithelium and shrinkage of glomeruli in kidney; and congestion of blood vessels in the brain with acute monocrotophos toxicity in chicken. Mahesh Kumar *et al.* (2003) observed the lesions of demyelination, degenerated and fragmented axons along with chromatolysis and neuronophagia in tri cresyl phosphate (TCP) treated White Leghorn hens.

Goyal (2002) observed the gross lesions such as degenerative and necrotic changes in liver, kidneys, heart and brain; and haemorrhages and oedema in lungs with anilofos toxicity in rats. The histopathological changes were hydropic degeneration, fatty changes and necrosis in liver; haemorrhages and oedema in lungs; degenerative and necrotic changes in kidneys; spongiform changes and proliferation of astrocytes in brain. Histopathological changes in liver observed in the present study may be due to liver damage caused by Kitazin. Liver being the primary organ involved in activation and detoxification of organophosphate esters is severely damaged by their toxicity.

Degenerative and necrotic changes in lymphoid organs indicate the immunosuppressive property of kitazin. The depletion of lymphoid cells in the lymphoid follicles is in conformity with the deceased TLC and lymphocyte count in the peripheral blood of Kitazin treated birds. The significantly deceased mean delta OD values of Con-A and LPS stimulated lymphocyte cultures in Kitazin treated birds also support the above findings. Histopathological examination of lymphoid organs is one of the important tools to assess, immunopathological effects of environmental contaminants. Since intestine almost always comes in contact with the compound through oral route of toxicity, the lesions in the intestine is due to damage caused by Kitazin. Menezes *et al.* (2017) reported that endosulfan induced apoptosis of chicken peripheral lymphocytes and splenocytes leading to immunotoxicity.

The histopathological changes in nervous tissue indicate the effect of accumulated acetylcholine following inhibition of acetylcholinesterase by Kitazin. This resulted in the neurological signs of toxicity observed in the present

study. The presumed mode of action of organophosphorous compounds is irreversible phosphorylation of neurotoxic esterase (NTE) and also acetylcholiesterase (AchE), and possible blockage of neural transmission. The covalent reaction between organophosphorous compounds and NTE, induces neuropathy. NTE enzyme is a specific protein, bound to neuronal membrane.

CONCLUSION

From the present study, it is concluded that Kitazin has adverse effects on general health, body weight and immune status of the birds. Kitazin induced effects are dependent on dose and duration of its exposure. Histopathological findings also confirm the immunosuppressive property even with low levels of exposure. In view of the above findings, adequate measures should be taken to minimize the indiscriminate use of Kitazin and its subsequent contamination of the environment.

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