Histopathological alterations induced by subacute imidacloprid toxicity in Japanese quails and its amelioration by Butea monosperma

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Abstract
Two weeks old Japanese quails (n=75) were divided into five groups comprising 15 birds in each. Group 1 served as control, group 2 was treated with imidacloprid @ 50 ppm in feed, group 3 was treated with B. monosperma @ 4 ppm, group 4 was treated with both imidacloprid @ 50 ppm and B. monosperma @ 2 ppm and group 5 was treated with imidacloprid @ 50 ppm and B. monosperma @ 4 ppm for 28 days. Liver of birds treated with imidacloprid showed severe degenerative changes and periportal necrosis and kidneys showed interstitial haemorrhages and vacuolar degenerative changes. Haemorrhages and disrupted bronchial epithelium in lungs, cellular oedema and degenerative changes in myofibers of heart, neuronal degeneration and vacuolation in brain, decreased lymphoid population in spleen, desquamation of villous epithelium in intestine, degeneration of the sciatic nerve fibers were evident in the imidacloprid treated birds. Co-administration of B. monosperma against imidacloprid restored gross and histopathological changes in these organs. This study indicated that, subacute exposure of imidacloprid @ 50 ppm in feed resulted in hepatotoxicity and cytotoxicity. Co-administration of B. monosperma ameliorated histological architecture of these organs with more pronounced effect at 4 ppm in feed.

Keywords: Butea monosperma, histopathology, imidacloprid, Japanese quails

Introduction
Imidacloprid (IMC) (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) was the first representative of neonicotinoid insecticides to be registered for use and is presently the most important commercial product because of its high efficacy against insects. Besides its agricultural use, it is also used to control houseflies on poultry farms. There is paucity of information available concerning the effects of IMC on animal health, as the insecticide that is likely to be used in future pest control programs. Human and animal exposure to imidacloprid is likely to occur during its use as an acaricide or an ectoparasiticide in animal houses and in poultry sheds. Increasing use of this insecticide and its potential toxicity among animals and humans warrants a heightened awareness about imidacloprid [1]. Few studies have been performed in mammals and birds with neonicotinoid insecticides and their relationship with oxidative effects in rats [2, 3]. Various herbs and herbal products are claimed to posses the antioxidant and hepatoprotective effects. Butea monosperma is being used in traditional medicine and found to have antimicrobial, wound healing, antifungal, anti diarrhoeal, hypoglycemic, hepatoprotective, antioxidant, antihelminthic, anti convulsive, antistress, anti-inflammatory activity [4, 5, 6]. It was found that these plants are found to posses polyphenolic constituents like flavonoids. Flavonoids are reported to have anti-inflammatory, antihepatotoxicity and antiulcer actions. They are potent antioxidants and have free radical scavenging abilities [7]. Present study was conducted to investigate the subacute imidacloprid toxicity and its effect on histopathology of major organs and to assess antioxidant role of Butea monosperma in imidacloprid-induced histopathological alterations.

Materials and methods
Chemical
Imidacloprid (Technical grade-C9H10ClN5O2; 97.20% by mass, 0.16% moisture content by mass and 0.035% acidity as H2SO4% by mass) was procured from Krishi Rasayan Export Pvt. Ltd., Samba, Jammu, India. All other chemicals used in the study were of analytical grade.
Plant
The leaves of *Butea monosperma* plant were collected from local region of Akola district of Mahrashtra, India in the month of January 2016. The botanical identity was confirmed by the expert taxonomist Dr. S.P. Rothe, Professor and Head, Department of Botany, Shri Shivaji Science College, Akola (M.S.). The leaves were shade dried and mixed with the feed in a powdered form.

Experimental birds
The study was conducted in two weeks Japanese quails (n=75) which were procured from Venkteshwara Hatcheries, Pune. The birds were maintained under standard management conditions and were provided with *ad libitum* feed and water. Before the start of experiment, the birds were acclimatized for a period of 7 days. The experimental trial was approved by the Institutional Animal Ethics Committee of the institute.

Experimental design
Birds were randomly divided into five equal groups comprising 15 birds in each. Group 1 served as control, group 2 was treated with imidacloprid @ 50 ppm in feed, group 3 was treated with *B. monosperma* @ 4 ppm in feed, group 4 was treated with both imidacloprid @ 50 ppm and *B. monosperma* @ 2 ppm in feed and group 5 was treated with imidacloprid @ 50 ppm and *B. monosperma* @ 4 ppm in feed for a period of 28 days. Birds were sacrificed at the end of 28 days to study gross and histopathological alterations. At necropsy, organs such as liver, kidney, lung, heart, brain, spleen, intestine, sciatic nerve were thoroughly examined and gross lesions were carefully recorded. Tissue pieces of representative organs were preserved in 10% buffered formalin. After proper fixation, tissues were processed by paraffin embedding technique. Tissue sections were cut at 4-5 μ thickness and stained with routine hematoxylin and eosin (H & E) stain for detailed histopathological examination.

Results
The gross lesions observed in the liver clearly indicated the damage. The gross changes were appreciable only in the liver tissue, while other organs did not show gross pathological alterations. The detailed histopathological examination revealed following pathological alterations.

Liver: Grossly, liver of birds treated with imidacloprid was congested, fragile with pale discoulouration. Histopathologically liver showed severe degenerative changes in hepatocytes with bile duct hyperplasia (Plate 1) and perportal necrosis (Plate 2), dilatation of sinusoidal space, mild to moderate congestion. The liver of group 3 birds did not show any histological changes. Supplementation of *Butea monosperma* ameliorated gross and histopathological changes in imidacloprid treated birds (Group 4 and 5). The intensity of damage was less compared to group 2 birds with mild degenerative changes and congestion with more pronounced effect in group 5.

Kidneys: Severe congestion, interstitial haemorrhages and vacuolar degeneration in tubular and glomerular epithelium with disruption of basement membrane (Plate 3), Shrinkage of glomeruli, proximal tubular necrosis, epithelial damage and loss of nuclei, mononuclear cell infiltration and RBCs in interlobular vein (Plate 4) were evident in kidney sections of imidacloprid treated birds. Co-treatment with *Butea monosperma* (group 4 and 5) restored these histological alterations in the kidney tissues in which only mild degenerative changes along with interstitial haemorrhages, leucocytic infiltration in the interstitium with congested interlobular vein were evident (Plate 5). The kidney of group 1 and 3 birds showed no histological alterations.

Lungs: Severe congestion of blood vessels and haemorrhages along with severe alveolar infiltration of mononuclear cells (Plate 6), disruption of bronchial epithelium (Plate 7). Haemosiderin laden macrophages were characteristic findings in the lungs of birds treated with imidacloprid. Co-administration of *Butea monosperma* to the imidacloprid treated birds (group 4 and 5) showed improvement in the lung tissue histology with milder changes indicating partial amelioration. The lung of birds of control group and that of group 3 showed normal histology.

Heart: Heart of imidacloprid treated birds revealed mild cellular oedema, mild to moderate degenerative changes in myofibers along with haemorrhages and leucocytic infiltration (Plate 8). However, birds given imidacloprid along with *B. monosperma* revealed mild degenerative changes in the cardiac muscle fibers (Plate 9) indicating reparative quality on cardiac tissue by *B. monosperma*.

Brain: Congestion, degeneration of neurons, nuclear migration, vacuolation, oedema, haemorrhages in parenchyma and gliosis were evident in the imidacloprid treated birds (Plate 10). These lesions were of mild intensity in the birds of groups 4 and 5 but were less pronounced in group 5. Brain of group 1 and 3 birds showed no histological alterations.

Spleen: Decreased lymphoid population with congestion of red pulp (Plate 11) and fibrous tissue proliferation (Plate 12) were evident in the spleen of imidacloprid treated birds. These perturbations were not pronounced in groups 4 and 5 (Plate 13). The spleen of birds in control group and that of group 3 showed normal histology.

Intestine: Distortion and desquamation of villous epithelium with broadening of villi (Plate 14), increased cellularity and degenerated intestinal glands/ crypts of Liberkuhns were evident in sections of intestine from imidacloprid treated group (Plate 15). However, treatment with *B. monosperma* ameliorated histological lesions of intestine maintaining normal intestinal glands in groups 4 and 5 (Plate 16). This effect was more profound in birds of group 5.

Sciatic nerve: Histopathology of sciatic nerve from group 2 birds showed focal areas of demyelination and degeneration of the nerve fibers (Plate 17), axonal degeneration and areas of infiltration with mononuclear inflammatory cells. However, these changes were mild in groups 4 and 5 (Plate 18) indicating amelioration by *B. monosperma*.
Plate 1: Section of liver from T2 group showing bile duct hyperplasia (× 40) H & E

Plate 2: Section of liver from T2 group showing dilated sinusoids along with periportal necrosis. (× 400) H & E

Plate 3: Section of kidney from T2 group showing vacuolar degenerative changes in tubular and glomerular epithelium with disruption of basement membrane. (× 400) H & E

Plate 4: Section of kidney from T2 group showing haemorrhages in interstitium and RBCs in interlobular vein. (× 100) H & E

Plate 5: Section of kidney from T5 group showing mild degenerative changes with haemorrhages in interstitium and RBCs in interlobular vein. (× 100) H & E

Plate 6: Section of lung from T2 group showing haemorrhages with infiltrating mononuclear cells. (× 100) H & E

Plate 7: Section of lung from T2 group showing disruption of bronchial epithelium (× 100) H & E

Plate 8: Section of heart from T2 group showing degenerative changes in muscle fibers. (× 100) H & E
Plate 9: Section of heart from T5 group showing comparatively mild degenerative (× 100) H & E changes in the muscle

Plate 10: Section of brain from T2 group showing neuronal degeneration, edema and gliosis at few places. (× 100) H & E

Plate 11: Section of spleen from T2 group showing decreased lymphoid population with congestion in red pulp (× 100) H & E

Plate 12: Section of spleen from T2 group showing proliferation of connective tissue (× 100) H & E

Plate 13: Section of spleen from T4 group showing congestion in white pulp and mild depletion of lymphocytes (× 100) H & E

Plate 14: Section of intestine from group T2 showing desquamated villous epithelium with broadening of villi (× 100) H & E

Plate 15: Section of intestine from T2 group showing degenerated intestinal glands/Crypts of Liberkuhn (× 400) H & E

Plate 16: Section of intestine from group T4 showing restoration of villous epithelium (× 100) H & E
study are in agreement with findings of other researchers [11, 12]. Treatment with imidacloprid treated quails and its amelioration by co-exposure to imidacloprid. Further, severe histological changes observed in the kidney included interstitial haemorrhages and vacuolar degeneration in tubular and glomerular epithelium with disruption of basement membrane which again revealed toxic nature of imidacloprid. It could be due to increased glomerular filtration and capillary permeability by imidacloprid toxicity as a result of leakage of proteins that causes tubular necrosis. Similarly, lung also showed haemorrhages along with severe alveolar infiltration by leucocytes with demyelinated nerve fibers and leucocytic infiltration. (× 400) H & E

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Discussion
Histopathological findings of severe degeneration and necrosis of hepatocytes with dilatation of sinusoidal space are in agreement with Eissa (2004) who reported severe dilated portal spaces, large degenerated area, faintly stained cells and nuclei in liver histological changes in liver after 3 and 6 weeks of treatment with imidacloprid @ 1/50th of LD50 in Japanese quails [9]. One researcher also reported similar changes in liver of layer chickens given single oral dose of imidacloprid @ 139 mg/kg [10]. Hepatic necrosis might be due to oxidative stress induced by imidacloprid that further involved in the cellular protein degradation. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells. Histopathological changes observed in the kidney included interstitial haemorrhages and vacuolar degeneration in tubular and glomerular epithelium with disruption of basement membrane which again revealed toxic nature of imidacloprid. It could be due to increased glomerular filtration and capillary permeability by imidacloprid toxicity as a result of leakage of proteins that causes tubular necrosis. Similarly, lung also showed haemorrhages along with severe alveolar infiltration by leucocytes with demyelinated nerve fibers and leucocytic infiltration. (× 400) H & E

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References
11. Nellore KJ, Doss, Chimata MK. Histopathological studies

Plate 17: Section of sciatic nerve from T2 group showing demyelinated nerve fibers with leucocytic infiltration. (× 400) H & E

Plate 18: Section of sciatic nerve from T5 group showing mildly degenerated myelin sheath. (× 400) H & E

~ 256 ~
17. Sharma N, Shukla S. Hepatoprotective potential of aqueous extract of Butea monosperma against CCl4 induced damage in rats. Experimental and Toxicologic Pathology 20011; 63(7, 8):671-676.