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A Review: Pathological studies on imidacloprid toxicity and its amelioration with vitamin C

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Abstract
Imidacloprid (IMC), the first neonicotinoid insecticide causes almost complete and virtually irreversible blockage of post-synaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects. The IMC intoxication leads to exhibition of clinical signs such as depression, decreased appetite, excessive salivation, watery diarrhea, muscle weakness and ataxia. IMC causes congestion and hemorrhages in visceral organs such as liver, kidney, lung, spleen, intestine, pancreas and heart. Histopathological lesions due to IMC intoxication are congestion, hemorrhages, necrosis of hepatocytes, fatty change along with lymphocyte infiltration in portal triad area and hyperplasia of bile duct in liver, bronchiolitis, myocarditis, depletion of lymphocytes in lymphoid organs, interstitial nephritis and degeneration of Purkinje cells in cerebellum. Vitamin C acts as a co-factor in various enzymatic reactions and most important free radical scavenger. Vitamin C supplementation resulted in noticeable amelioration of clinical and pathological lesions of the IMC toxicity.

Keywords: Imidacloprid, Vitamin C, lesions, toxicity

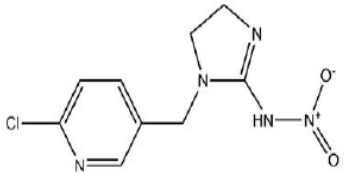
Introduction
Imidacloprid, the first neonicotinoid insecticide (Moriya *et al.*, 1992) [1] which is considered most likely alternative to organophosphate insecticide. Imidacloprid was introduced in the market in 1991 and in India in 1997-98. Chemically, it is 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine and presently the most widely used insecticide against a broad spectrum of sucking and chewing pests (Jeschke *et al.*, 2010) [2]. Imidacloprid causes almost complete and virtually irreversible blockage of post-synaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous system (CNS) of insects. Globally, the sale of imidacloprid as insecticide is growing fastly because it has high selectivity for insects and apparently safe for humans (Matsuda *et al.*, 2001) [3]. Although imidacloprid has low mammalian toxicity but its accidental or careless applications have been reported to cause health hazards in many non-target organisms such as fish, aquatic invertebrates, birds, animals and humans (Nagata *et al*., 1999) [4].
Vitamin C is hydrophilic and most important free radical scavenger in extra-cellular fluids thus; it traps the free radicals in the aqueous phase and protects the biomembranes from per oxidative damage (Sulak *et al.*, 2005 [5]; Uzunhisarcikli *et al.*, 2007 [6]).

Imidacloprid Toxicity
Imidacloprid, presently the most widely used insecticide because of its high efficacy against insects and low soil persistence (Chao and Casida, 1997) [7]. Imidacloprid is used to kill sucking insects, some chewing insects including termites, soil insects, and fleas on pets. In addition to its topical use on pets, imidacloprid may be applied to structures, crops, soil, and as a seed treatment (Tomlin, 2006 [8]; Fossen, 2006 [9]). The important physical and chemical properties of imidacloprid are summarized in Table 1.
Imidacloprid acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system (Buckingham *et al.*, 1997 [10]; Matsuda and Sattelle, 2005 [11]). In insects, these receptors are located only within the central nervous system. Following binding to the nicotinic receptor, nerve impulses will spontaneously discharge at first, followed by failure of the neuron to propagate any signal (Schroeder and Flattum, 1984 [12]; Sheets, 2001 [13]). However, binding affinity of imidacloprid to the nicotinic receptors in mammals and birds is much less than that of insect nicotinic receptor (Tomizawa and Casida, 1999 [14]). Imidacloprid can enter in body via oral, dermal and inhalation routes. More than 90% of its excretion occurs within 24 hours and thus very less residual effect (Klein and Karl, 1990 [15]; Broznić *et al.*,

2008 [16]). Hepatotoxicity is the primary effect observed in the imidacloprid toxicity (Kammon *et al*., 2010) [17] however; oxidative stress (El-Gendy *et al.,* 2010) [18], nephrotoxic (Kammon *et al.*, 2010 [17]) and immunotoxic (Kammon *et al*., 2012 [19]) effect of this compound have been reported. Hussein *et al*. (2014) [20] noticed the malformations and teratogenic effects on chick embryos after the treatment of chicken eggs with 10µg, 15µg, 25µg, and 40µg doses of imidacloprid. Imidacloprid also caused growth retardation, retraction of yolk sac, head enlargement, ectopia viscerale and many other developmental defects in chick embryos.

LD50 is the amount (dose) of a compound that is lethal to one-half (50%) of the animals exposed to it under defined conditions like species, route of exposure and duration of exposure (Sandhu and Brar, 2009 [21]). In mice, LD50 values had estimated at 130 mg/kg for males and 170 mg/kg for females (Thyssen and Machemer, 1999 [22]). LD50 in Japanese quails has been reported 31 mg/kg body weight (Eissa, 2004 [23]). However, the LD50 of imidacloprid in chicken has reported to be 104.1 mg/kg body weight (Kammon *et al.*, 2010 [17]).

Table 1: Physical and chemical properties of imidacloprid

Property	Information	References
Molecular weight	255.69 g/mol	HSDB (2006) [24]
Odor	Slight characteristic order	HSDB (2006) [24]
Physical state	Crystalline solid	Tomlin (2006) [8]
Melting point	144^0C	HSDB (2006) [24]
Color	Colorless crystals	HSDB (2006) [24]
Solubility in water	0.61 g/L at 20 ºC	Tomlin (2006) [8]
Chemical name	IUPAC name: 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine C.A. name: 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine	Tomlin (2006) [8]
Chemical formula and structure	 $C_9H_{10}ClN_5O_2$	HSDB (2006) [24]

Clinical signs of toxicity

Thyssen and Machemer (1999) [22] reported that very high dose of imidacloprid orally in rats exhibited clinical signs such as lethargy, vomiting, diarrhea, salivation, muscle weakness and ataxia, which were all indicative of imidacloprid action on nicotinic receptors. Other signs of exposure at high doses were uncoordinated gait, tremors, and reduced activity.

Bhardwaj *et al.* (2010) [25] observed that 90 days oral toxicity of imidacloprid in female rats with doses of 0, 5, 10, 20 mg/kg/day caused decrease in the body weight gain at 20 mg/kg/day and at necropsy, the relative body weights of liver, kidney and adrenal were also significantly increased at this dose level. No mortality occurred during the treatment period while decrease in food intake had noticed at high dose level.

Kammon *et al.* (2010) [17] observed that administration of imidacloprid at the dose of 139 mg/kg orally, within 15 minutes, produced signs of toxicity like sluggishness, closed eyes/dropped eyes, chickens sitting on hocks, open mouth breathing, muscular tremors, chickens lying on one side and walking when forced to do so but lying down again. Some of chickens also revealed paralysis and watery diarrhea. Deaths occurred within 5-24 hours following insecticide administration.

Bagri *et al*. (2013) [26] revealed that 110mg/kg body weight oral administration of imidacloprid in Swiss albino male mice did not cause any significant effect on body weight, relative organs weight and morphometric measurements of various body organs in mice.

Pathological Studies

USEPA (1998) [27] observed that in a sub chronic oral toxicity of imidacloprid at concentrations of 150, 600 and 2400 parts per million (ppm) in Wistar rats for a period of 13 weeks produced hypertrophy of hepatocytes and sporadic necrosis of hepatocytes in liver.

Eissa (2004) [23] observed that Japanese quails treated with 1/50 LD50 of imidacloprid for 3 and 6 weeks showed degenerative changes in liver. The author also reported that after 3 weeks of recovery period liver revealed irregular arrangement of hepatocytes with abnormal architecture, large area of necrosis, dilated sinusoidal spaces, large and small areas of degeneration with faintly stained cytoplasmic nuclei. Microscopically, testis revealed degenerative changes included bizarre cells, disappearance of spermatogenetic cells, devoid of sperms in the tubules and thickened tunica albuginea. After 3 weeks recovery period no recovery signs observed but debris of sperms, vacuolation of spermatocytes and rupture of basement membrane noticed.

Jain *et al*. (2004) [28] observed that imidacloprid toxicity in rats caused focal interstitial pneumonia characterized by thickening of alveolar wall and aggregation of lymphoid follicles around the bronchioles in lungs, congestion and cloudy swelling in liver and mild hemorrhages in heart. However they inferred that imidacloprid toxicity in rats was mild in nature.

Bhardwaj *et al.* (2010) [25] observed that 90 days oral toxicity of imidacloprid in female rats with 20 mg/kg/day caused a mild focal necrosis of hepatocytes with swollen nuclei in liver. Kidney showed degeneration of tubules and glomerulus. Cerebellum of brain showed degenerative changes in Purkinje cells and loss of granules in granular layer.

Kammon *et al*. (2010) [17] observed that administration of imidacloprid at the dose of 139 mg/kg orally produced some hemorrhages and paleness of the kidneys. Microscopically, liver tissue of intoxicated chickens showed congestion, hemorrhages, degeneration of hepatocytes, coagulative necrosis and mild dilation of hepatic sinusoids. Kidneys showed sub-capsular hemorrhages and vacuolar degeneration

of tubular epithelial cells as well as focal coagulative necrosis. Mohany *et al.* (2012) [29] observed that treatment of male albino rats with imidacloprid at the rate of 0.21 mg/ kg body weight for 28 days orally produced histopathological changes in liver like homogenous cytoplasm in hepatocytes, congested central vein, leucocytic infiltration and fibroblasts around the bile duct and central vein.

Soujanya *et al.* (2012) [30] studied that administration of imidacloprid at the rate of 80 mg/kg body weight per day through oral gavage for 28 days resulted in neuro toxicity characterized by marked congestion in cerebellum, degeneration of Purkinje cells with loss of dendrites, vacuolation around neurons, shrunken neurons, chromatolysis and ultra structural alterations like vacuolar mitochondria, apoptotic nuclei with disrupted and margination of chromatin material.

Soujanya *et al.* (2013) [31] observed that administration of imidacloprid at 80 mg/kg body weight/day for 28 days revealed vacuolation, degeneration of seminiferous tubules, detachment of germinal cells from basement membrane, increased interstitial spaces, disrupted basement membrane, presence of very few leydig cells, severe congestion in interstitial spaces and tunica albuginea of testis. Ultra thin sections of testis in imidacloprid treated group showed swollen nucleus, increased perinuclear space, varied size and shape of mitochondria and degeneration of spermatids.

Soujanya *et al.* (2013) [32] observed that administration of imidacloprid at 80 mg/kg body weight/day by oral gavage for 28 days in male rats resulted in marked dilation, congestion of central vein, portal vein and sinusoidal spaces, vacuolation/fatty change and degenerated hepatocytes in liver. Ultra thin sections of the liver revealed swollen nuclei, varied size and shape of mitochondria, disrupted chromatin and rough endoplasmic reticulum.

Kumar *et al.* (2014) [33] observed that administration of doses of 25, 50 and 75% LD50 imidacloprid orally in female albino mice caused degeneration of hepatocytes, dilation of sinusoids, irregular hepatic cords arrangement, leucocytes infiltration, necrosis and hemorrhages in liver. The severity of lesions was more as the dose of imidacloprid increased.

Vitamin C Supplementation

Vitamin C acts as a co-factor in various enzymatic reactions, including several collagen synthesis reactions. It is important in wound healing and in preventing bleeding from capillaries. Vitamin C may also act as an antioxidant and protect against oxidative stress.

Eissa (2004) [23] reported that Japanese quail treated with 1/50 LD50 of imidacloprid for 3 and 6 weeks along with supplementation of vitamin C @ 0.08 mg/kg body weight showed a protective effect against the imidacloprid toxicity on liver and testis of Japanese quails.

Georgieva and Popov (2007) [34] reported genotoxic effects of imidacloprid using rabbit peripheral blood lymphocytes and a dose dependent increase in sister chromatid exchanges (SCE) per cell. This genotoxic effect was found to be protected by simultaneous use of vitamin C and vitamin E alongwith imidacloprid.

Zaahkook *et al*. (2009) [35] found amelioration in serum lactate dehydrogenase, alanine transaminase and alkaline phosphatase activities after a recovery period of 3 weeks in Japanese quails treated with 1/50 LD50 of imidacloprid plus vitamin C @ 0.08 mg/kg body weight as compared to birds treated with imidacloprid alone. They also observed insignificant alterations in levels of serum total protein, albumin and globulin in birds treated with imidacloprid in combination with vitamin C.

El-Gendy *et al*. (2010) [18] studied that oral administration of imidacloprid at the rate of 14.9 mg/kg body weight in male mice produced oxidative stress, which was evident from increased levels of lipid peroxidation, activities of the antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione-s-transferase and decreased levels of reduced glutathione. Whereas Vitamin C (200 mg/kg body weight) treated mice showed ameliorating effect documented by decreased levels of lipid peroxidation.

Kammon *et al*. (2011) [36] studied the protective effect of vitamin C against chlorpyrifos chronic toxicity in broilers. Oral administration of vitamin C @ 100 mg/kg body weight partially ameliorated the degenerative changes in kidney and heart produced by treatment with 0.8 mg/kg body weight (1/50 LD50) of chlorpyrifos. There was insignificant alteration in serum glucose, creatinine, creatine kinase, uric acid, total protein, albumin and cholesterol level and activities of alanine transaminase, aspartate transaminase and alkaline phosphatase and hemoglobin, total leukocyte count and differential leukocyte count when vitamin C had supplemented with chlorpyrifos treatment. It was concluded that supplementation of vitamin C diminished the severity of lesions produced by chronic chlorpyrifos toxicity in broilers.

Soujanya *et al.* (2013) [31] reported that exposure to imidacloprid at 80 mg/kg body weight for 28 days in male rats induced histological and ultrastructural alterations in spermatids of testis and these changes were moderately ameliorated due to supplementation of vitamin C in imidacloprid treated rats.

Soujanya *et al.* (2013) [32] observed that administration of imidacloprid at 80 mg/kg body weight/day by oral gavage for 28 days in male rats along with supplementation of vitamin C caused significantly ($P \leq 0.05$) reversal of the imidacloprid-induced changes.

Conclusion

Imidacloprid causes various clinical signs and pathological lesions in animals. Vitamin C supplementation resulted in noticeable amelioration of clinical and pathological lesions of the toxicity. Vitamin C supplementation also leads to insignificant alterations in various biochemical parameters.

References

1. Moriya K, Shibuya K, Hattori Y, Tsuboi SI, Shiokawa K, Kagabu S. 1-(6-Chloronicotinyl)-2-nitroimino-imidazolidines and related compounds as potential new insecticides. Bioscience, biotechnology, and biochemistry. 1992; 56(2):364-5.
2. Jeschke P, Nauen R, Schindler M, Elbert A. Overview of the status and global strategy for neonicotinoids. Journal of agricultural and food chemistry. 2010; 59(7):2897-908.
3. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M, Sattelle DB. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends in pharmacological sciences. 2001; 22(11):573-80.
4. Nagata K, Aoyama E, Ikeda T, Shono T. Effects of nitenpyram on the neuronal nicotinic acetylcholine receptor-channel in rat phaeochromocytoma PC12 cells. Journal of Pesticide Science. 1999; 24(2):143-8.

5. Sulak O, Altuntas I, Karahan N, Yildirim B, Akturk O, Yilmaz HR, Delibas N. Nephrotoxicity in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamins E and C. Pesticide Biochemistry and Physiology. 2005; 83(1):21-8.
6. Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A, Buyukkomurcu F. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pesticide biochemistry and physiology. 2007; 87(2):115-22.
7. Chao SL, Casida JE. Interaction of imidacloprid metabolites and analogs with the nicotinic acetylcholine receptor of mouse brain in relation to toxicity. Pesticide Biochemistry and Physiology. 1997; 58(1):77-88.
8. Tomlin CDS. The Pesticide Manual. (14th Edn.). In: British Crop Protection Council, Alton, Hampshire, UK, Toxicology. Kreiger R. (Edt.). Academic Press. 2006; 11:23-30.
9. Fossen M. Environmental fate of imidacloprid. California Department of Pesticide Regulation, 2006, 1-6.
10. Buckingham S, Lapied B, Corronc HL, Sattelle F. Imidacloprid actions on insect neuronal acetylcholine receptors. Journal of experimental biology. 1997; 200(21):2685-92.
11. Matsuda K, Sattelle DB. Mechanism of selective actions of neonicotinoids on insect nicotinic acetylcholine receptors, 2005.
12. Schroeder ME, Flattum RF. The mode of action and neurotoxic properties of the nitromethylene heterocycle insecticides. Pesticide Biochemistry and Physiology. 1984; 22(2):148-60.
13. Sheets LP. Imidacloprid: A Neonicotinid Insecticide. In: Handbook of Pesticide Toxicology. (2nd Edn.). Krieger, R. I. (Edt.). Academic Press: San Diego, CA. 2001; 11:23-30.
14. Tomizawa M, Casida JE. Minor structural changes in nicotinoid insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors. British journal of pharmacology. 1999; 127(1):115-22.
15. Klein O, Karl W. Methylene-[14 C] Imidacloprid: Metabolism Part of the General Metabolism Study in the Rat. Bayer AG. Leverkusen-Bayerwerk, Germany, Study. 1990; (87264):51950-0021.
16. Broznić D, Marinić J, Tota M, Čanadi Jurešić G, Milin Č. Kinetic evaluation of imidacloprid degradation in mice organs treated with olive oil polyphenols extract. Croatica chemica acta. 2008; 81(1):203-9.
17. Kammon AM, Brar RS, Banga HS, Sodhi S. Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. Veterinarski arhiv. 2010; 80(5):663-72.
18. El-Gendy KS, Aly NM, Mahmoud FH, Kenawy A, El-Sebae AK. The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. Food and chemical Toxicology, 2010; 48(1):215-21.
19. Kammon AM, Brar RS, Banga HS, Sodhi S. Ameliorating effects of vitamin E and selenium on immunological alterations induced by imidacloprid chronic toxicity in chickens. J Environ Anal Toxicol S. 2012; 4:S4-007.
20. Hussein M, Singh V, Hassan M, Singh A, Yadav B. Malformations and teratogenic effects of imidacloprid on chick embryo. Sch J Appl Med Sci. 2014; 2:67-72.
21. Sandhu HS, Brar RS. Textbook of veterinary toxicology. (2nd Edn.) Kalyani Publishers, New Delhi-110 002, 2009.
22. Thyssen J, Machemer L. Imidacloprid: Toxicology and Metabolism. In: Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Yamamoto I, Casida JE (Edts.). Springer-Verlag: Tokyo, 1999, 213-22.
23. Eissa OS. Protective effect of vitamin C and glutathione against the histopathological changes induced by imidacloprid in the liver and testis of Japanese quail. The Egyptian Journal of Hospital Medicine. 2004; 16:39-54.
24. HSDB. Imidacloprid; National Institutes of Health, National Library of Medicine, U.S. Department of Health and Human Services, 2006, https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~LHDTca:1
25. Bhardwaj S, Srivastava MK, Kapoor U, Srivastava LP. A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. Food and chemical toxicology. 2010; 48(5):1185-90.
26. Bagri P, Kumar V, Sikka AK, Punia JS. Preliminary acute toxicity study on imidacloprid in Swiss albino mice. Veterinary World. 2013; 6(12):955-7.
27. USEPA. Imidacloprid, pesticide tolerance. Federal Register. 1998; 63(57):14363-14371.
28. Jain SK, Gupta RP, Punia JS. Pathological studies on imidacloprid toxicity in rats. Haryana Veterinarian. 2004; 43:42-4.
29. Mohany M, El-Feki M, Refaat I, Garraud O, Badr G. Thymoquinone ameliorates the immunological and histological changes induced by exposure to imidacloprid insecticide. The Journal of toxicological sciences. 2012; 37(1):1-11.
30. Soujanya S, Lakshman M, Anand Kumar A, Gopala-Reddy A. Histopathological and ultrastructural changes induced by imidacloprid in brain and protective role of vitamin C in rats. Journal of Chemical and Pharmaceutical Research. 2012; 4(9):4307-18.
31. Soujanya S, Lakshman M, Reddy AG. Protective role of vitamin C against the histopathological and ultrastructural changes induced by imidacloprid in testis of male rats. Int J Life Sci Biotechnol Pharma Res. 2013; 2:92-7.
32. Soujanya S, Lakshman M, Kumar AA, Reddy AG. Evaluation of the protective role of vitamin C in imidacloprid-induced hepatotoxicity in male Albino rats. Journal of natural science, biology, and medicine. 2013; 4(1):63-7.
33. Kumar A, Tomar M, Kataria SK. Effect of sub-lethal doses of imidacloprid on histological and biochemical parameters in female albino mice. ISOR J Environ Sci Toxicol Food Technol. 2014; 8:9-15.
34. Georgieva S, Popov B. A study on genotoxic effects of pesticide imidacloprid in rabbit peripheral blood lymphocytes by vitamins (C & E). Zhivotnovdni-Nauki. 2007; 44:50-4.
35. Zaahkook SA, Helal EG, Fahmy N, Al-Shinnawy MS, El-Ghany AB. Physiological study about imidacloprid toxicity and the role of vitamin" C" as a protective agent on Japanese Quails. Egyptian Journal of Hospital Medicine. 2009; 34:183-97.
36. Kammon AM, Brar RS, Sodhi S, Banga HS, Singh J, Nagra NS. Chlorpyrifos chronic toxicity in broilers and effect of vitamin C. Open veterinary journal. 2011; 1(1):21-7.