

CLINICO-PATHOLOGICAL STUDIES OF IMIDACLOPRID TOXICITY IN BROILER CHICKENS

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ABSTRACT

The maximum tolerated dose (MTD) and clinico-pathological studies on oral administration of imidacloprid (IMC) were undertaken in 40 broiler chicks of 14 days of age. The chicks were divided into eight groups (A to H) of five chicks each. The chicks were given IMC @ 52.05 (1/2 LD₅₀), 34.70 (1/3 LD₅₀), 26.03 (1/4 LD₅₀), 17.35 (1/6 LD₅₀), 13.01 (1/8 LD₅₀), 10.41(1/10 LD₅₀) mg/kg b. wt. by oral route in a single dose in groups A, B, C, D, E and F, respectively. IMC @ 29.74 mg/kg b. wt. (1/3.5 LD₅₀) orally in a single dose was administered in group G. Based on mortality pattern, MTD was calculated as 26.03 mg/kg b. wt. To verify MTD, additional group (group H) of five chicks of 14 days of age were given 26.03 mg/kg b. wt. of IMC orally. The birds administered IMC at a higher dose than MTD exhibited nervous clinical signs such as tremors of head and paralysis of one or both the legs. Other abnormalities such as abduction of legs from body and belly of bird touching the ground, ataxia, loss of appetite, respiratory distress, excessive salivation, drooping of wings, diarrhoea and closing of eyes were also seen. Necropsy examination of birds of all the IMC treated groups revealed severe congestion in various organs such as liver, kidney, lungs and spleen. Besides this hydropericardium and blood filled pouches on the surface of liver were also noticed. Mild hemorrhage was also noticed on heart, liver and lungs. Histopathologically, liver revealed severe congestion, hemorrhages, fatty changes and focal area of necrosis accompanied with infiltration of lymphocytes and heterophils. Mild pericarditis and myocarditis, peribronchiolar infiltration of lymphocytes and congestion in other organs such as spleen, kidney and thymus were also seen. It was concluded that MTD of IMC in chickens at 14 days of age was 26.03 mg/kg b. wt. by oral route and the doses higher than MTD caused severe toxicity in broiler chickens.

Key words: Imidacloprid, maximum tolerated dose, toxicity

Indiscriminate use of insecticides poses major threat to the health of animals and human beings. Indirect exposure of insecticides to the birds occurs using insecticide contaminated poultry litter e.g. rice hulls and wood shavings (Amure and Stuart, 1978) and/or through usage of insecticide contaminated feed ingredients for poultry ration (Naber, 1977). Imidacloprid (IMC) is most widely used neonicotinoids insecticide in agriculture (Whitehorn *et al.*, 2012). In addition, it is used to control houseflies in poultry farms (Kammon *et al.*, 2010).

Experimental toxicity studies require determination of maximum tolerated dose (MTD) or median lethal dose (LD₅₀). The LD₅₀ of IMC in chicken is reported to be 104.1 mg/kg b. wt. (Kammon *et al.*, 2010). However, MTD of IMC in chicken has not been determined yet. Estimation of MTD values of IMC in chicken will be valuable for conducting further experimental toxicity studies. In present study, MTD of IMC was calculated and clinico-pathological effects were studied in broiler chickens following oral administration of IMC.

MATERIALS AND METHODS

Experimental Birds: Forty, day-old broiler chicks were

procured from a local hatchery. The chicks were reared in the departmental animal house under strict hygienic conditions. The birds were provided feed and water *ad libitum*. The temperature of animal house was maintained between 21-31°C throughout the experiment. The approval of Institutional Animal Ethical Committee was obtained before the use of chicks in this study.

Imidacloprid: IMC (Confidor 17.8% SL - Bayer Crop Science Limited) was used in the present study.

Determination of MTD: MTD of IMC was determined after screening various doses of IMC with respect to oral LD50 which has been reported as 104.1 mg/kg body weight (BW) (Kammon *et al.*, 2010) following the standard method (Moser and Padilla, 1998). Out of total 40 chicks, thirty chicks of 14 days of age were divided into six groups (A-F) with each group containing five chicks. The birds in these different groups were given IMC @ 52.05 (1/2 LD₅₀), 34.70 (1/3 LD₅₀), 26.03 (1/4 LD₅₀), 17.35 (1/6 LD₅₀), 13.01 (1/8 LD₅₀), 10.41(1/10 LD₅₀) mg/kg b. wt., respectively by oral route as a single dose (Table 1). Based on mortality pattern observed, five birds of 14 days of age (Group G) were administered IMC @ 29.74 mg/kg b. wt. (1/3.5 LD₅₀) orally as a single dose. Further, to verify MTD, an additional group (group

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Table 1**Dose of imidacloprid (IMC) and mortality due to its oral administration in different groups of broiler chicken**

Group	Number of chicks	Dose of IMC (mg/ kg b. wt.; orally) with respect to oral LD ₅₀	Number of chicks died/number of chicks administered with IMC
A	5	52.05 (1/2 LD50)	3/5 (60%)
B	5	34.70 (1/3 LD50)	2/5 (40%)
G	5	29.74 (1/3.5 LD50)	1/5 (20%)
C	5	26.03 (1/4 LD50)*	0/5
D	5	17.35 (1/6 LD50)	0/5
E	5	13.01 (1/8 LD50)	0/5
F	5	10.41 (1/10 LD50)	0/5

*MTD of imidacloprid: 26.03 mg/kg b. wt. orally

H) of five chicks of 14 days age were given orally with 26.03 mg/kg b. wt. of IMC.

Toxicity Study: Per-acute toxicity study was undertaken in different groups (A to G) after administration of different doses of IMC using the oral LD₅₀ as a baseline dose. Clinical signs and mortality in each group were recorded. A detailed necropsy of dead or sacrificed birds from each group was conducted after 24 h of IMC administration. Representative pieces of liver, heart, lung, kidney, spleen and thymus were collected in 10% buffered formalin for histopathological studies.

RESULTS AND DISCUSSION

Determination of MTD: Mortality recorded in different groups is shown in Table 1. In group A, one bird died after three hours and two more birds died after 15 h post treatment. Mortality in groups B and G was of two and one birds, respectively after 16 h of post treatment. No mortality was noticed in any other groups. MTD is the maximum dose, which can be tolerated by birds without showing any adverse effects. Therefore, maximum dose that the bird could tolerate was 26.03 mg/kg b. wt. (Group C). Therefore, MTD of IMC in broiler chicks was found to be 26.03 mg/kg b. wt. by oral route at day 14 of age. Oral LD₅₀ of IMC has been reported (104.1 mg/kg b. wt.) but there seems to be no report in the literature on the MTD of IMC in chicken.

Clinical Signs: The birds of groups A, B and G exhibited nervous signs such as tremors of head that started to occur episodically after 6-10 min of IMC administration and lasted for 1 to 10 seconds. Paralysis of one or both the legs along with posture abnormality showing legs abducted from body and belly of bird touching the ground were also seen (Fig. 1). Other clinical signs observed were loss of appetite, respiratory distress, excessive salivation, dropping of wings, diarrhea, ataxia and closing of eye. These clinical signs were more severe in groups A, B and G as compared to groups C and D. However, such clinical signs were not noticed in groups E and F.

The severity of these clinical signs decreased progressively and the signs completely disappeared within 24 h of administration of IMC in the surviving birds. The birds with doses higher than MTD showed slow respiration rate, however, just before death the rate of respiration became high. The nervous signs and high respiration rate could be correlated with the agonist action of IMC at nicotinic acetylcholine receptors (nAChR) which has been reported to induce neuromuscular paralysis (Tomizawa and Casida, 2005). The early appearance (within 10-15 min of administration) and completely disappearance (within 24h of administration) of clinical signs might be associated with the prompt absorption and excretion of imidacloprid (Broznic *et al.*, 2008).

Pathological Lesions: Necropsy examination of the birds that died or were sacrificed in different IMC treated groups revealed severe congestion in various organs such as liver, intestine, kidney (Fig. 2), lungs and spleen. The congestion was less severe in groups C, D, E and F as compared to the groups A, B and G. Besides this hydropericardium, blood filled pouches on the surface of liver were also noticed in group A. Mild hemorrhages were also noticed on heart, liver and lungs.

Histopathologically, liver revealed severe congestion, hemorrhages, fatty changes (Fig. 3) and focal area of necrosis accompanied with infiltration of lymphocytes and heterophils. The liver plays central role in the biotransformation and disposition of xenobiotics (Murray *et al.*, 1999) and has been reported to be the principal target organ of IMC toxicity (Sheets, 2001; Kammon *et al.*, 2010). In lungs, there was severe congestion along with hyperplasia of bronchus associated lymphoid tissue (BALT) (Fig. 4). Heart revealed the presence of mild pericarditis (Fig. 5) characterized by hemorrhages and infiltration of heterophils. Mild hemorrhages and infiltration of few heterophils were also noticed in myocardium (Fig. 6). Other organs such as spleen, kidney and thymus revealed mild to moderate congestion. Nephrotoxic lesions due to IMC toxicity has also been reported by other workers in chickens (Kammon *et al.*, 2010) and rats



Fig 1. (Group A): Chick showing paralyzed right leg and belly of bird touching the ground



Fig 2. (Group B): Congestion in kidneys

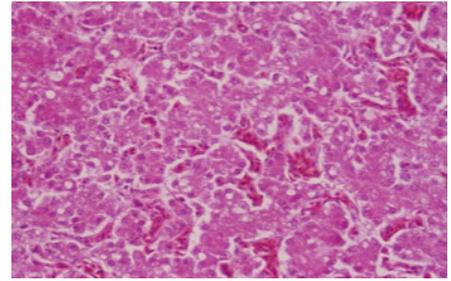


Fig 3. (Group B): Section of liver revealing severe congestion and fatty changes (H&E × 400)

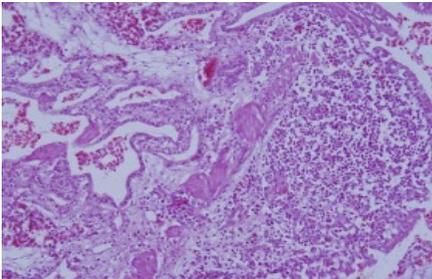


Fig 4. (Group A): Section of lung revealing severe congestion along with hyperplasia of bronchus associated lymphoid tissue (BALT). (H&E × 200)

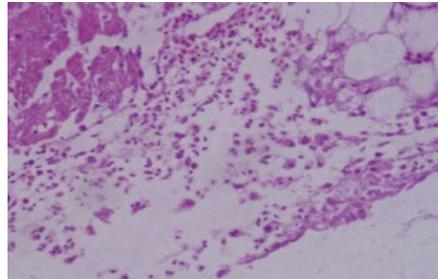


Fig 5. (Group A): Section of heart revealing pericarditis characterized by infiltration of heterophils and hemorrhages (H&E × 400)

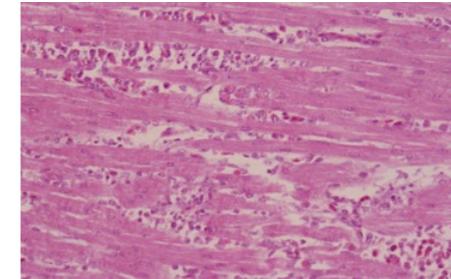


Fig 6. (Group B): Section of heart revealing myocarditis characterized by infiltration of heterophils and fragmented myocardial fibers (H&E × 400)

(Bhardwaj *et al.*, 2010). Nevertheless, the kidney plays important role in the detoxification for many xenobiotics and is frequently susceptible to the nephrotoxic effects (Kammon *et al.*, 2010). These results are in accordance with the findings of other workers who have studied the toxicity of IMC in chickens (Kammon *et al.*, 2010), quails (Omiamia, 2004) and rats (Bhardwaj *et al.*, 2010; Mohany *et al.*, 2012; Soujanya *et al.*, 2013) and these workers have also reported degeneration, necrosis and vascular disturbances due to administration of IMC. It is concluded from present study that acute toxicity of imidacloprid is causes circulatory disturbances, hepatotoxicity and nephrotoxicity in broiler chickens.

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