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Research Article

ORAL SUB-CHRONIC THIACLOPRID TOXICOSIS IN POULTRY BIRDS (*GALLUS DOMESTICUS*): A CLINICO-BIOCHEMICAL EVALUATION

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ABSTRACT

The present experiment was undertaken to investigate the effect of oral sub-chronic toxicity of thiacloprid at concentration of 1 mg/kg/day for 90 consecutive days on clinical signs and biochemical parameters of birds. The clinical signs observed were of mild to moderate severity. There was significant elevation in the levels of plasma aminotransferases, alkaline phosphatase and lactate dehydrogenase. Thiacloprid did not significantly alter the levels of plasma acid phosphatase, whole blood cholinesterase and total plasma proteins. It produced significant hyperglycemia and hypercholesterolemia. There was also significant elevation in the levels of renal parameters after repeated oral exposure of thiacloprid. The repeated oral toxicity on thiacloprid suggested that it is moderately risk insecticide in *Gallus domesticus*.

Keywords: Biochemical alteration, *Gallus domesticus*, Sub-chronic toxicity, Thiacloprid

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Pesticides are frequently used for controlling weeds, pests, in forestry, public health, domestic premises and agriculture (Aktar *et al.*, 2009). Its contamination poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms, to insects, plants, fish, and birds (Brown, 2004). Thiacloprid is a neonicotinoid insecticide that belongs to a new group of active ingredients, the cyanoamidines, and is effective on contact and via stomach action. It produces its action by binding agonistically to the nicotinic acetyl cholinergic receptors (nAChRs) in the CNS of insects (Liu and Casida, 1993 and Zhang *et al.*, 2000). Poultry meat and eggs forms an important constituent of diet world over, owing to its relatively lower cost and superior nutritional value (Hussain *et al.*, 2015). Exposure of poultry to pesticides often results in numerous health hazards and economic losses, in addition to posing a threat to public health, due to the presence of pesticide residues in poultry meat and eggs (Hisashi *et al.*, 2006). Since there are very few reports on toxicological aspects of this compound in poultry and data generated in one animal species cannot be extrapolated in other species. The present experiment was undertaken to investigate the effect of 90 days oral administration of doses of thiacloprid on clinical symptoms and biochemical parameters of birds.

MATERIALS AND METHODS

The present study was conducted on one and half year old layer poultry birds (*Gallus domesticus*). The birds were procured and housed at the Layer House of the Poultry Farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. The birds were provided with standard feed and clean water *ad libitum* and were acclimatized to the layer house for ten days prior to the commencement of the study at ambient temperature of 25 °C and 45-55% relative humidity, with 12 h each of dark and light cycles. The experimental protocol for general procedure and use of poultry birds for conducting the present study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, GADVASU, Ludhiana via no. VPS.08/63-71 dated 14.01.2008.

Chemicals: Technical grade thiacloprid (Alanto 240 SC, Thiacloprid 21.7%), was commercially obtained from the authorized dealer of Bayer Cropscience Limited, Sabarkanta, Gujarat. All chemicals used in this study were of high purity. Based on the literature available, a suitable non-lethal dose of thiacloprid i.e. 1 mg/Kg/day was selected for the administration by oral route (NRAAVC, 2001).

Experimental design: Fifty two birds were randomly divided into ten groups. The requisite amount of insecticide as per body weight was dissolved in 10 ml of water and administered directly into the proventriculus of the bird by using catheter with 2 ml glass syringe and/or tuberculin syringe as per given plan. Groups I to IV consisted of 4 birds in each group were kept as healthy control in which distilled water was administered and no insecticide was given. The groups V, VI, VII, VIII, IX and X contained six birds each in which thiacloprid was given @ 1 mg/kg/ day for 15, 30, 45, 60, 75 and 90 days, respectively. The birds were starved overnight and their body weights were recorded before the treatment. All the birds were weighed after 15 days and dosage of thiacloprid corrected according to changes in body weights. Thiacloprid exposed birds were kept under close observation to monitor

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the nature, degree and time of occurrence of various signs of toxicity and mortality, if any, during the experimental period.

Estimation of various parameters: Blood was collected in heparinised vials directly by cardiac puncture on 0, 30, 60 and 90 treatment days from Groups I, II, III and IV birds, respectively and on 0, 15, 30, 45, 60, 75 and 90 days from groups V, VI, VII, VIII, IX and X birds, respectively for biochemical analysis. Plasma was separated immediately after collection by centrifugation at 2500 rpm for 15 min at room temperature, used immediately for biochemical analysis or kept in deep freeze for subsequent analysis. The whole blood cholinesterase activity was measured according to the method of Tecles and Ceron (2001). All the biochemical parameters were analyzed using standard autopak kits (Bayer Diagnostics India Ltd., Baroda, Gujarat).

Statistical analysis: Results were expressed as Mean ± SEM. Statistical significance was determined by one way analysis of variance (ANOVA). The treatment groups were compared with control group using Tukey's HSD post-hoc test for multiple comparisons with SPSS 16.0 for computer programme. The values of P less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Daily oral administration of 1 mg/kg/day of thiacloprid produced mild to moderate signs of toxicity in poultry birds as depicted in Fig. 1, 2, 3 and 4. The birds in the control groups of I, II, III and IV remained normal and did not show any of the visible toxic signs and change in body weight as observed in the birds of all treatment groups. There was significant decrease in body weight of birds kept in group VII after 30 days of exposure to thiacloprid which continued till 90 days of treatment in groups VIII, IX and X (Table 1). Avery *et al.* (1993) and Werner *et al.* (2010) reported that the reduction in intake of food may be due to repellent effects of the pesticides, whereas, Li *et al.* (2007) reported that food intake reduction could be attributed to the toxic effects of pesticides that lead to less food intake and resulted in the loss of body weight. Similar results were obtained after repeated oral administration of imidacloprid at 20 mg/kg/d during 90 days exposure which produced significant decrease in food consumption and body weight gain of rats together with significant toxicity symptoms (Bhardawaj *et al.*, 2010). Significant decrease in spontaneous locomotor activity in the birds treated with the thiacloprid indicated the accumulation of thiacloprid or its metabolites in the brain. Chao and Casida (1997) reported the accumulation of imidacloprid in mouse brain following direct intraperitoneal administration. Neonicotinoids are neuroactive insecticides which act on nicotinic acetylcholine receptors (nAChR) on the post synaptic membrane, disrupting neural transmission in the central nervous system of insects. This can lead to sub-lethal effects, such as paralysis or even the death of the animal (Simon-Delso *et al.*, 2015).

Repeated oral administration of thiacloprid at dose

Table 1

Effect of sub-chronic oral administration of thiacloprid (1 mg/kg/day) on toxic signs and body weight (kg) in poultry birds (*Gallus domesticus*)

	Time (Days)						
	Treatment						
Groups	0	15	30	45	60	75	90
	Toxic Signs						
Control*	No visible toxic signs	-	No visible toxic signs	-	No visible toxic signs	-	No visible toxic signs
Thiacloprid**	No visible toxic signs	Slight dullness	Reduced feed intake, slight dullness, watery undigested faeces	Ruffled feathers and slight cyanotic comb	Lethargy, decreased locomotor and rearing activity	Drooping head, decreased limb grip strength, incordinated gait, inability to fly and put weight on legs	Off- feed, severe limb weakness
	Body Weight (Mean ± SE)						
Control*	1.39±0.05^{a}	-	1.33±0.08^{a}		1.37±0.10^{a}	-	1.42±0.09^{a}
Thiacloprid**	1.53±0.07^{a}	1.42±0.05^{ab}	1.32±0.03^{bc}	1.30±0.04^{bc}	1.27±0.02^{bc}	1.25±0.06^{bc}	1.22±0.04^{c}

* 4 (number of birds), **6 (number of birds); Mean with at least one common superscript are similar within the group (P<0.05)

Table 2

Effect of sub- chronic ral administration of thiacloprid (1 mg/kg/day) on blood biochemical enzymatic parameters

	Time (days)						
	Treatment						
Groups	0	15	30	45	60	75	90
Aspartate Aminotransferase (U/L)							
Control*	127.75±12.26^{a}	-	121.00±9.25^{a}	-	120.25±11.33^{a}	-	125.00±6.70^{a}
Thiacloprid**	123.33±7.85^{a}	137.0±10.98^{a}	144.17±8.16^{ab}	161.40±6.52^{bc}	165.67±4.99^{bc}	187.00±5.32^{c}	217.17±7.28^{d}
Alanine Aminotransferase (U/L)							
Control*	6.25±0.25^{a}	-	8.75±1.31^{a}	-	8.00±1.08^{a}	-	9.50±0.96^{a}
Thiacloprid**	10.33±0.80^{a}	13.17±0.48^{a}	14.50±0.62^{ab}	15.60±0.75^{bc}	16.50±0.56^{cd}	18.60±0.38^{de}	19.50±0.85^{e}
Alkaline Phosphatase (U/L)							
Control*	197.75±8.37^{a}	-	207.25±12.89^{a}	-	215.25±9.25^{a}	-	217.25±2.43^{a}
Thiacloprid**	203.17±4.85^{a}	221.33±5.61^{ab}	230.83±6.51^{ab}	249.00±9.80^{b}	280.00±8.56^{c}	316.00±10.77^{d}	336.33±8.98^{d}
Lactate Dehydrogenase (U/L)							
Control*	394.00±16.31^{a}	-	403.50±10.81^{a}	-	410.50±14.38^{a}	-	424.00±9.17^{a}
Thiacloprid**	405.67±31.08^{a}	459.62±13.05^{a}	478.33±36.85^{ab}	482.60±24.17^{ab}	435.50±16.74^{ab}	550.20±18.95^{bc}	619.17±24.03^{c}
Blood Glucose (mg/dl)							
Control*	177.75±15.11^{a}	-	169.25±18.40^{a}	-	173.50±3.88^{a}	-	159.25±16.08^{a}
Thiacloprid**	172.17±9.02^{a}	197.83±11.40^{ab}	209.00±10.93^{b}	216.80±9.11^{bc}	233.17±15.56^{bc}	249.60±8.05^{cd}	261.00±4.15^{d}
Plasma Cholesterol (mg/dl)							
Control*	112.50±3.01^{a}	-	126.25±5.27^{a}	-	116.00±6.24^{a}	-	119.00±2.83^{a}
Thiacloprid**	128.33±12.80^{a}	126.33 ±9.93^{a}	143.17±8.99^{ab}	163.20±11.40^{ab}	171.33±5.82^{bc}	181.80±2.96^{bc}	200.33±4.46^{c}
Blood Urea Nitrogen (mg/dl)							
Control*	1.43 ±0.13^{a}	-	1.70±0.28^{a}	-	1.63±0.10^{a}	-	1.78±0.23^{a}
Thiacloprid**	1.62±0.24^{a}	1.93±0.19^{ab}	2.18±0.31^{bc}	2.50±0.37^{bc}	2.78±0.28^{c}	2.88±0.16^{cd}	2.90±0.15^{d}
Creatinine (mg/dl)							
Control*	0.34±0.03^{a}	-	0.38±0.04^{a}	-	0.39±0.0^{a}	-	0.48±0.10^{a}
Thiacloprid**	0.29±0.02^{a}	0.31±0.03^{a}	0.38±0.01^{ab}	0.48±0.04^{bc}	0.60±0.05^{cd}	0.69±0.04^{d}	0.84±0.06^{e}

* 4 (number of birds), **6 (number of birds); Mean with at least one common superscript are similar within the group ($P<0.05$)

of 1 mg/kg/day for 90 consecutive days produced significant elevation in the levels of both plasma aspartate aminotransferase (AST) and plasma alanine aminotransferase (ALT) in poultry birds. The levels of AST and ALT were significantly enhanced in groups VII, VIII, IX and X at 45, 60, 75 and 90 days of exposure with thiacloprid, respectively as compared to the control groups at same time intervals (Table 2). Kaur (2005) also reported elevation in the levels of both AST and ALT following repeated oral administration of imidacloprid (0.5 mg/kg/day) for 150 days in cow calves. The chickens given imidacloprid at rate of 139 mg/kg body weight via oral gavages showed significant increase in the activities of serum ALT and AST (Kammon *et al.*, 2010). The elevation of ALT and AST in blood may be indicative of cellular injury or damage of the hepatic parenchymal cells and other vital tissues including cardiac tissue (More, 2006). Although damage to any particular organ cannot be cited as a cause of increased level of aminotransferases, the moderate increase in AST and ALT levels in blood in the present study suggested mainly hepatocellular injury or altered hepatocellular membrane permeability.

There was no significant alteration in the plasma levels of acid phosphatase enzyme (ACP) but a significant elevation in the levels of plasma alkaline phosphatase (ALKP) was seen from day 45 till day 90 in the thiacloprid treated poultry birds groups as compared to control group (Table 2). Similar changes in the levels of plasma phosphateses in cow calves and chicken, respectively were also found after oral administration of imidacloprid (Kaur, 2005 and Kammon *et al.*, 2010). Alkaline phosphatase is distributed in liver, bone, intestinal mucosa, lungs,

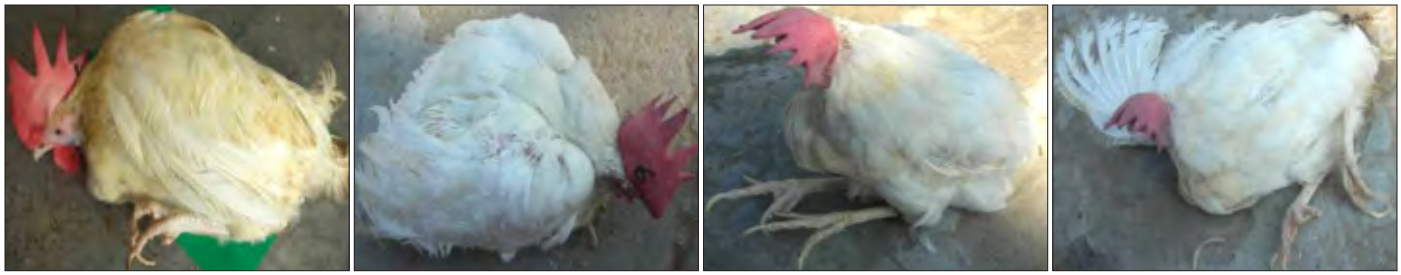

Fig. 1-4. (1) Bird showing slight dullness at day 30 in group VII. **(2)** Bird showing depression and mild cyanosis of comb at day 45 in group VIII. **(3)** Bird sitting on its haunches and unable to put weight on limbs at day 75 in group IX. **(4)** Bird showing paralysis, ruffled feathers and inability to stand and fly at day 90 in group X.

placenta and kidney (Sheets, 2001). USEPA (2003) has documented that liver is the main target organ in thiacloprid toxicity; therefore, the high alkaline phosphatase levels in the present study might have occurred due to its harmful effects on hepatocytes.

There were no significant alterations in the levels of whole blood cholinesterase (ChE) enzyme in any of the treated groups (Table 2). The observed findings are in agreement with Kaur (2005) and Sheets (2001) in cow calves and experimental adult wistar rats, respectively, where repeated oral administration of imidacloprid produced no inhibition of cholinesterase activity in plasma, brain and erythrocytes. It is in contrast to study in which it was observed that 90 days oral toxicity of imidacloprid in female rats with 20 mg/kg/day could cause decrease in serum and brain acetyl choline esterase (AChE) (Bhardwaj *et al.*, 2010). There were significant alterations in the levels of lactate dehydrogenase from day 75 as compared to control group (Table 2). LDH is an intracellular enzyme, which is widely distributed throughout the body and is found at high levels in tissues that utilize glucose for energy. It is a non-specific indicator of tissue injury and is not related to any specific organ. LDH levels may also be increased whenever there is cell necrosis or when there is neoplastic proliferation of cells (More, 2006).

The blood glucose levels significantly started rising from day 30 of treatment with thiacloprid till 90 days as compared to control groups. There was significant rise in the levels of plasma cholesterol from 60 days onwards in groups VIII, IX and X (Table 2). The chickens given imidacloprid @ 139 mg/kg body weight showed significant increase in the glucose levels (Kammon *et al.*, 2010). The results of the present investigation are in agreement with that of hyperglycemia induced by toxicants that has been related to the increased secretion of catecholamines from adrenal medulla following stress conditions, which stimulates glycogenolysis within hepatocytes (Cowell, 2004). Hypercholesterolemia was also reported in cow calves after administration of imidacloprid for 150 days (Kaur, 2005). Alterations in cholesterol levels are not liver specific but elevated level of total cholesterol and its ester can be observed in animals and birds having bile duct occlusion (Cowell, 2004).

There was no significant alteration in the levels of total plasma proteins (Table 2). The findings are in agreement with the studies in rats, chicken and cow calves, respectively, after the administration of imidacloprid (Premlata, 2002; Kammon *et al.*, 2010 and Kaur, 2005). The levels of total plasma proteins were lower in mice fed thiacloprid for 14 weeks and were higher in rats fed thiacloprid upto 13 weeks (NRAAVC, 2001). Although liver plays vital role in protein metabolism yet total protein concentration is of little value in assessment of liver functions (Brar *et al.*, 2000).

Table 2 shows a significant elevation in the levels of blood urea nitrogen. The levels increased significantly from 1.62 ± 0.24 mg/dl (0 day) to 2.90 ± 0.15 mg/dl (90 day) of administration of thiacloprid. The observed findings are similar to the study by USEPA (2003) in which significant increase in the levels of blood urea nitrogen was reported following daily administration of thiacloprid for 10 weeks in dogs. Similar results were obtained after 90 days oral toxicity of imidacloprid in female rats with 20 mg/kg/day (Bhardawaj *et al.*, 2010). Increase in blood urea nitrogen is seen in shock, congestive heart failure, dehydration and renal causes like obstruction in urinary passage (Brar *et al.*, 2000). The plasma creatinine levels were significantly elevated from pre-exposure level of 0.29 ± 0.02 to 0.84±0.06 mg/dl at the end of experiment (Table 2). Results are similar to the findings of Kaur (2005) who reported increase in plasma creatinine after administration of imidacloprid in cow calves. The present findings, however, are in contrast to the work when rats were administered imidacloprid for 28 days, a non-significant change in levels of plasma creatinine were reported (Premlata, 2002). Increase in creatinine levels indicates renal tubular necrosis and nephron damage (Brar *et al.*, 2000).

The clinical and biochemical studies revealed that thiacloprid produced time dependent toxicosis in poultry birds. Thiacloprid is moderately toxic to poultry birds (*Gallus domesticus*), can be used in recommended concentration but the birds should not be exposed for longer duration.

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