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Immune Response to Sub Acute Toxicity of Thiacloprid insecticide in *Gallus domesticus*

Saloni Singla* and V. K. Dumka

Department of Veterinary Pharmacology and Toxicology,
Guru Angad Dev Veterinary and Animal Science University, Ludhiana
*salonivpt@gmail.com

Abstract

Thiacloprid is fairly a new chemical, but it has established itself as key component in insecticides because of its selectivity and low toxicity. It is effective on contact and via stomach action and bind agonistically to the nicotinic acetylcholine receptors in the CNS of insects, affecting synaptic transmission and leading to disruption of the nervous system. The immune system is a host defense system comprising many biological structures and processes within an organism that protects against disease. It can be the target of many chemicals, with potentially severe adverse effects on the host's health. The health implications of these immune dysfunctions are increased risk of infectious diseases; development of neoplasia; autoimmune disorders and allergies. Estimation of total immunoglobulins and enumeration of B and T lymphocytes in blood after repeated oral administration of thiacloprid at the dose rate of 10 mg/kg/day revealed no significant alterations in Gallus domesticus. The observed findings in the present study indicated that repeated thiacloprid exposure in poultry birds did not adversely affect the immune status of Gallus domesticus and thus is immunologically safer insecticide

Keywords: Toxicity, *Gallus domesticus*, Thiacloprid, Insecticide, immune system.

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Introduction

In the last couple of decades, the insecticide market was dominated by four major chemical classes - organochlorines, organophosphates, carbamates and pyrethriods, all of which act as neurotoxicants. Their ability to control insects, however, diminished with years due to their extensive toxicity potential and because the pests developed resistance towards them [1]. Therefore, there arises a constant need to develop new compounds with wider spectrum of activity, low toxicity potential to non-target species and different mechanism of action. In this regard, several newer groups including formamidine and neonicotinoid insecticides were developed.

Thiacloprid, a novel neonicotinoid insecticide, was developed by Bayer AG and Nihon Bayer Agrochem and first registered in Brazil in 1999. United State Environment Protection Agency has placed technical thiacloprid in toxicity category II for acute oral administration and category III for dermal application and inhalation studies [2]. Because of its selectivity for insects [3], thiacloprid is thought to be safer than other pesticides [4], and it now has the highest production of all insecticides worldwide [5]. It is known to act as a nicotinic acetylcholine receptor (nAChR) agonist [3] and binds to and activates nAChR at its nicotine and acetylcholine binding site, affecting synaptic transmission and leading to disruption of the nervous system [6]. The potential

* *Author for correspondence*

risks from thiacloprid have gained significant attention due to its worldwide use and persistence in crops. Laboratory studies of it have shown genotoxicity in both rats [7] and cultured human lympho-cytes. [8] Moreover, neurobehavioral deficits were found in rats exposed to neonicotinoids in the uterus [9]. The immune system is a frequent target organ of toxic insult following chronic or acute exposure to environmental chemicals, therapeutic drugs, abused drugs or radiation. Due to its importance in assessing drugs and nondrug chemicals, immunotoxicity testing is required by many regulatory agencies [10]. Although there is a large body of literature addressing immune responses during insecticide exposure, the immunotoxicity of Thiacloprid is poorly understood. Therefore, this study aimed to increase our understanding of the immunotoxic effects of sub acute Thiacloprid exposure.

Material and method

The present study was conducted on one and half year old layer *Gallus domesticus*. The birds were procured and housed in pens 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. The thiacloprid (Alanto 240 SC, Thiacloprid 21.7%), used in this study was commercially obtained from the authorized dealer of Bayer Cropscience Limited, Sabarkanta, Gujarat. Based on the recommended concentrations of thiacloprid used for crop protection, a suitable non-lethal dose of thiacloprid was selected for the administration by oral route. All animal procedures were performed in accordance with the guidelines for the care and use of experimental animals of the Committee for Purpose of Supervision of Experiments on Animals (CPCSEA).

Thirty six birds were randomly divided into seven groups. Group I, II and III containing four birds each served as control in which no insecticide was administered. Groups IV, V, VI and VII contained six birds each and were administered thiacloprid in repeated oral dose of 10 mg/kg/day for 7, 14, 21 and 28 days or till the birds were sacrificed or dead, respectively. The requisite amount of insecticide was suspended in water and administered directly into proventriculus of the bird by using a catheter with 2 ml glass syringe and/or tuberculin syringe. The birds were starved overnight and their bodyweights were recorded before the start of administration of thiacloprid. All the birds were weighed weekly and doses of thiacloprid corrected according to changes in their body weights.

To study the immunological parameters, blood samples were collected directly by cardiac puncture on 0, 14 and 28 treatment days from Groups I, II and III of the birds, respectively and on 0, 7, 14, 21 and 28 days in the birds of treatment groups IV,V,VI and VII, respectively. EDTA containing blood was enumeration of B and T cells and blood containing no anticoagulant for estimation of total immunoglobulins. For collection of serum, blood samples were collected in non-heparinized test tubes and were allowed to clot at room temperature. The serum separated from clot was then centrifuged at 2500 rpm for 15 min and clear supernatant serum was collected for estimations.

Two widely used procedures for measuring immunoglobulin levels are single radial immunodiffusion (RID) [11] and zinc sulfate (ZnSO4) turbidity. The latter is a "salting out" procedure that depends on the biochemical properties of immunoglobulin in relation to the characteristics of ZnSO4. In this study, this technique was performed as previously described [12,13]. Lymphocytes were separated by centrifuging whole blood on premix commercial preparation of Ficoll (Histopaque, Sigma Ficoll- Paque, Pharmacia) as described by Boyum (1968) [14]. Separation of T and B lymphocyte was done by Nylon wool column separation method as described by Julius *et al* (1973) [15]. 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.

Result and Discussion

Repeated oral administration of thiacloprid at a dose rate of 10 mg/kg/day in *Gallus domesticus* had no significant effect on the total immunoglobulins (Table 1). The observed findings are not in agreement with work of

Table 1. Effect of sub-acute oral administration of thiacloprid (10 mg/kg/day) on total immunoglobulins (g/100ml) and B- and T-lymphocytes (%) in *Gallus domesticus*.

Time (Days)	0	7	14	21	28
Total Immunoglobulins (Mean ± S.E)					
Control	1.67±0.06	-	1.70±0.10	-	1.68±0.13
Thiacloprid	1.54±0.08	1.77±0.06	1.64±0.07	1.53±0.05	1.47±0.07
B-Lymphocytes (Mean ± S.E)					
Control	3.50±1.0	-	2.25±0.7	-	2.50±0.4
Thiacloprid	2.33±1.3	2.83±1.0	2.53±1.4	2.17±0.6	3.18±1.0
T-Lymphocytes (Mean ± S.E)					
Control	19.25±1.3	-	17.75±2.6	-	18.25±1.9
Thiacloprid	17.67±1.3	18.33±1.1	17.34±1.2	16.67±1.4	16.17±0.8

Street and Sharma (1995) [16] who reported decrease in total immunoglobulin levels due to DDT, carbaryl and carbofuran in rabbits but are in agreement with Goyal and Sandhu (2012) [17] where thiacloprid at dose rate of 1 mg/kg/day for 90 consecutive days also did not produce any significant alterations in the levels of total immunoglobulins in *Gallus domesticus*. Disorganization of endoplasmic reticulum and reduction in the total immunoglobulin synthesis has been reported due to lindane in rats and sheep [18, 19] and endosulfan in birds [20]. Nicotine is also reported to decrease serum immunoglobulin levels [21].

Immunoglobulins are the gamma globulins produced by B cells in the bursa of fabricus in chicken. The antibody producing B cells have well developed rough endoplasmic reticulum where the immunoglobulins are synthesized [22]. Chemicals usually affect the protein synthesis required for cell proliferation as well as immunoglobulins. Chemicals that have potential for alkylating nucleic acids or proteins are considered immunotoxic because they interfere with functions of various proteins in the immune response. Exposure to pesticides generally leads to tissue damage associated with alterations in body proteins and immune response [23]. However, no significant change in total immunoglobulin content in the present study might be due to insignificant effect of thiacloprid on peripheral lymphocytes which synthesize immunoglobulins and with resultant no functional impairment of lymphocytes [20].

Subacute oral exposure of thiacloprid @ 10 mg/kg/day for 28 consecutive days had no significant effect on the count of B and T-lymphocytes (Table 1). The observed findings were similar to the results obtained after repeated oral administration of thiacloprid at the dose rate of 1 mg/kg/day in birds [17]. In contrast to above findings, Tamang *et al* (1988) [24] observed significant decrease in number of B lymphocytes in cypermethrin treated groups. Ingestion of permethrin is also reported to reduce the ability of T-lymphocytes to recognize and respond to foreign proteins with doses equivalent to 1/100 of the LD_{50} inhibiting T-lymphocytes over 40 percent [25]. In mice, organophosphate insecticides are reported to induce immunosuppression [26] and DDT, mirex and endosulfan reduce both the B and T lymphocytes [27].

B cells are lymphocytes that are produced and matured in bursa of fabricus and are precursors of humoral immunity. The principal functions of B cells are to make antibodies against antigens and then they produce their effect through antibodies [28]. The T lymphocytes are also produced in bursa of fabricus but mature in thymus. They are the precursors of cell mediated immunity in chicks as they attack the invaders directly [29]. Depletion of these cells is usually associated with change in number of T and B cells and thus immunity of the animal.

The decreased number of lymphocytes along with their decreased functional capacity has been reported following pesticide intoxication [30]. The immunosuppressive action of pesticides mainly occurs due to their detrimental effect on lymphoid organs. As the

number of circulating lymphocytes in peripheral blood is considered an index of functional ability of lymphoid organs and thus the immunogenic response in birds and mammals [27], the non-significant effect of thiacloprid on B and T lymphocytes in the present study suggested that the neonicotinoid insecticide has no adverse effect on the immunocompetence of *Gallus domesticus*.

The observed findings in the present study indicated that sub acute thiacloprid exposure in poultry birds did not adversely affect the immune status of *Gallus domesticus* and thus is immunologically safer insecticide.

Conflict of Interest

The authors declare no potential conflicts of interest that could inappropriately bias the contents of this manuscript

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