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RESEARCH ARTICLE

GROSS MORPHOLOGICAL CHANGES IN CHICK EMBRYOS AFTER EXPOSURE TO NEONICOTINOID INSECTICIDE IMIDACLOPRID

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ABSTRACT

Introduction: Imidacloprid is one of the major representatives of the new generation of neonicotinoid insecticides derived from nicotine isolated from the tobacco plant. Imidacloprid is a widely applied pesticide due to their higher affinity for insect nicotinic acetylcholine receptors, it acts on nervous system. Worldwide, it is considered to be one of the insecticides used in the largest volume. Pesticide substances are biologically active and must be tested to ensure that their use will not give rise to any unacceptable risks to non-target organisms (i.e. humans, animals, plants and environment).
Methods: The current study was carried out on 400 fertile eggs of white leghorn chicken obtained from government poultry farm after taking permission from animal ethical committee. Chicken eggs exposed to Imidacloprid with doses of 2.5µg, 5µg, 10µg and 20µg in a volume of 2.5µl, 5µl, 10µl and 20µl respectively and control same as test group. The embryos were terminated on 21st day, eggs shell broken with a scalpel and embryos removed. Gross morphological changes observed and recorded.
Results: The results show that experimental group had comparatively more cases of morphological changes growth retardation resulting into failure of retraction of yolk sac, limbs deformities, beak deformities, head enlargement and ectopia viscerale as compared to controls.
Conclusion: Imidacloprid exposure increases the risks of morphological changes with increasing embryonic age. Comparatively higher doses proved more toxic and also caused many morphological changes and developmental defects.

Key Words: Imidacloprid, Chick Embryos and Morphological Changes.

INTRODUCTION

Pesticides are widely used in food production systems and in agriculture sectors of some of the countries because of their increased food demands. Also, a large number of benefits have been derived from the use of pesticides in public health, forestry and domestic sphere. Many other kinds of benefits which are often going unnoticed by general public may be attributed to the use of pesticides. Today, more than 800 products of pesticides are in regular use. The extensive uses of pesticides are widely used to enhance the crop production and other benefits and have raised concerns about potential adverse effects on the environment, human health and non-target animals. Imidacloprid (IMC) is one of the major representatives of the new generation of neonicotinoid insecticides. It was patented for the first time in 1985 by Bayer and was placed on the market in 1991. Imidacloprid is a systemic chloro-nicitnyl insecticide. It was the first member of a new family, the neonicotinoids, and is chemically related to the nicotinic acetylcholine receptor agaonists nicotine and epibatidine. Imidacloprid was discovered in 1984 at Nihon Bayer Agrochem in Japan by screening novel synthetic compounds for a high affinity to the insect nicotinic AChRs receptors, but with low toxicity to vertebrate species (Kagabu S. 1997). Imidacloprid interacts with the acetylcholine receptor, which is widely conserved across species (Seifert J *et al*., 2005). In the past few years the agricultural production has been enormously enhanced by the use of many synthetic pesticides. Although, their application is based on selective toxicity for certain organisms yet it has resulted in serious effects on many non-target organisms as well. The use of pesticides has created a type of chemical environment which is proving harmful to the living systems. As the Food and Agriculture Organisation of the United Nations (FAO) defined, pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, including vectors of human or animal disease or weed which can cause harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs (FAO 2002).

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MATERIALS AND METHODS

The present study was carried out in the department of Anatomy Govt. Medical College, Ambedkar Nagar and Santosh Medical College Ghaziabad U.P. on 400 fertile eggs of

Table 1. Gross morphological changes on 21st day in chick embryos after exposure to Neonicotinoid insecticide Imidacloprid

S. No.	Morphological Changes	Number of embryos in which abnormality is detected											
		Group A			Group B			Group C			Group D		
		Study (2.5µL IMC) (n=50)	Control (2.5µL NS) (n=50)	p-value	Study (5.0µL IMC (n=50)	Control (5.0µL NS) (n=50)	p-value	Study (10.0µL IMC) (n=50)	Control (10.0µL NS) (n=50)	p-value	Study (20.0µL IMC) (n=50)	Control (20.0µL NS) (n=50)	p-value
1	Growth retardation	18	0	-	24	0	-	25	1	<0.001*	35	2	<0.001*
2	Head enlargement	4	0	-	5	1	<0.001*	5	0	-	10	3	0.071*
3	Limb deformities	11	0	-	11	0	-	15	2	0.001	15	1	<0.001*
4	Beak deformities	7	0	-	6	0	-	10	2	0.027	16	1	<0.001*
5	Ectopia viscerale	4	0	-	7	0	-	16	0	-	26	0	-
6	Scanty feather	8	0	-	12	1	<0.001*	14	0	-	19	1	<0.001*
7	Failure of retraction of yolk sac	39	5	<0.001	33	5	<0.001	34	7	<0.001	40	6	<0.001

Table 2. Lethal effects after exposure to Neonicotinoid insecticide Imidacloprid in chick embryos

	Lethal effects											
	Group A			Group B			Group C			Group D		
	Study (2.5µL IMC)	Control (2.5µL NS)	p-value	Study (5.0µL IMC)	Control (5.0µL NS)	p-value	Study (10.0µL IMC)	Control (10.0µL NS)	p-value	Study (20.0µL IMC)	Control (20.0µL NS)	p-value
Number of fertile eggs used	50	50		50	50		50	50		50	50	<0.001*
Number of dead embryos (%)	12 (24%)	2 (4%)	0.007*	15 (30%)	2 (4%)	0.001*	19 (38%)	5 (10%)	0.002*	23 (46%)	4 (8%)	

Figure:1 Shows Imidacloprid exposed chick with failure of retraction of yolk sac(YS) in "A" Test group (T) and Control (C) normal.

white leghorn chicken weighing between 35 to 55 grams (g) obtained from the government poultry farm after taking permission from animal ethical committee. Eggs from stock known to be nutritionally healthy were taken. Eggs were first candled in the order to discard the defective ones and to outline the exact location of the air cell with a pencil. All the eggs were thoroughly washed with soap water solution and incubated immediately in standard electrical digital incubator (Micro Scientific Works Ltd.) with their broad end up where the chorioallantoic membrane is situated. The thermostat of the incubator will be set at temperature of 38° C in a humidity inside the chamber will be maintain at 60- 80 percent with no additional CO2 or O2 and the eggs were tilted three times a day.

Method for exposure to Neonicotinoid Insecticide Imidacloprid in chick embryos

Eggs will be candled on 2nd day to discard unfertilized eggs prior to exposure. Eggs were divided into four groups A, B, C and D. Each group has 50 eggs each. Control same as test group, treated with same volume of normal saline, whereas test group A, B, C and D were exposed to Imidacloprid with doses of 2.5µg, 5µg, 10µg and 20µg in a volume of 2.5µl, 5µl, 10µl and 20µl respectively and control same as test group on 2nd day of incubation. The solutions were taken in a tuberculin syringe. The broad end of the egg was wiped with a sterile gauze pad moistened with 70 percent alcohol solutions. A hole was drilled in eggshell in the centre of the surface over the air cell with a sterile needle; care was taken not to damage the shell membranes with point of drill. This is to avoid contact of air with the egg membrane. The needle was inserted horizontally into the air cell.

and the hardness of the tissue were measured. The dissection of chick embryo was done to observe the gross morphological changes and skeletal anomalies were carefully observed and photographs.

RESULTS

The chick embryo were examined for gross abnormalities and we observed Failure of Retraction of Yolk sac (figure 1, 2, 3), Growth Retardation (figure 2, 3,4,5 and 6), Limbs deformities (figure 7), Head Enlargement (figure 5), Ectopia Viscerale (figure 6) and Scanty feathers (figure 4) shown in table 1. In the control groups, either no morphological change, including growth retardation, head enlargement, limb deformities, beak deformities, ectopia viscerale, scanty feather and failure of retraction of yolk sac, was seen or if seen, the morphological changes were significantly less ($p<0.05$) than the respective study groups. In the study groups, significantly higher number of all deformities was seen. A chi-squared test for trend revealed a significantly higher ($p<0.05$) number of morphological changes with increasing doses of insecticides for Growth retardation, Beak deformities, Ectopia viscerale and Scanty feather. Imidacloprid causes gross morphological changes and developmental delays or smaller embryos after exposure to Neonicotinoid insecticide Imidacloprid. The effects of imidacloprid on growth retardation overall statistically significant for embryos at 10µg and 20µg levels. Imidacloprid had significant adverse effects on embryos failure of retraction of yolk sac although the control group has also shown the failure of retraction of yolk sac but the difference is statistically significant ($p<0.001$).

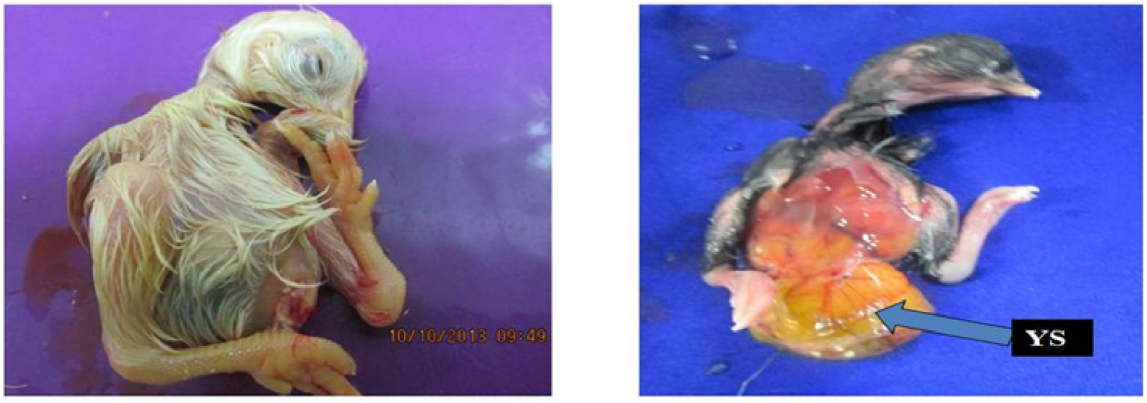

Figure:2 Shows Imidacloprid exposed chick with failure of retraction of yolk sac(YS) and growth retardation in "B" Test group (T) and control (C) is normal.

The needle was wiped with a sterile gauze pad between each injection and hole of the shell was sealed with Candle melted wax. After injection of drug, eggs were again kept for incubation at 38° C temperature. The embryos were terminated and eggs removed from the incubator on 21st day, the egg shell were broken with a scalpel and the embryos were removed. The number of live and dead embryos was noted. Parameters namely crown rump length; size and weight of the embryos

The lethal effects induced by Imidacloprid in chick embryo we found dead embryos in experimental A group 12(24%), B group 15(30%), C group 19(38%) and D group 23(46%). In control we observed in A group 2(4%), B group 2(4%), C group 5(10%) and D group 4(8%) embryos found dead shown in table 3. The mortality rate was 6.5% in control group and 34.5% in experimental group, this difference was statistically significant ($p<0.001$).

C T

Figure:3 Shows Imidacloprid exposed chick with failure of retraction of yolk sac(YS) and growth retardation in group "C" Test group (T) and control (C) is normal.

C T

Figure:4 Shows Imidacloprid exposed chick with growth retardation, Scanty feathers and beak deformity in "C" Test group (T) and control (C) is normal.

C T

Figure:5 Shows Imidacloprid exposed chick with Head enlargement(HE) and growth retardation in "C" Test group (T) and control (C) is normal.

Figure:6 Shows Imidacloprid exposed chick with growth retardation, Ectopia Viscerale (EV) in "C" Test group (T) and control (C) is normal

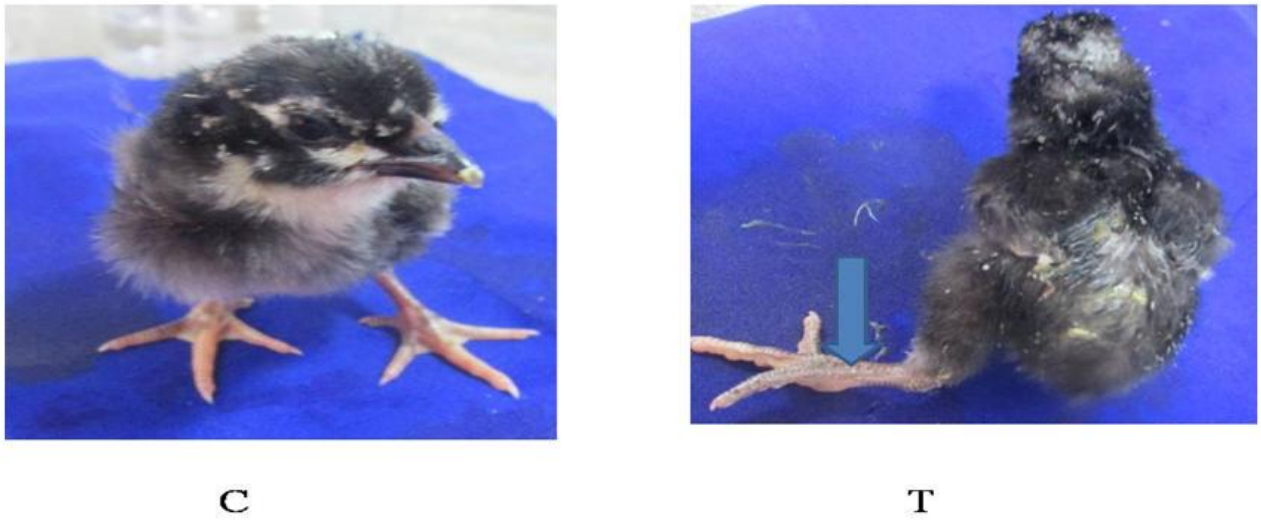

Figure:7 Shows Imidacloprid exposed chick limbs defects with arrow in "B" Test group (T) and control (C) is normal.

DISCUSSION

Imidacloprid is a neurotoxin that is selectively toxic to insects relative to vertebrates and most non-insect invertebrates. Imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding or partial binding to specific areas of the nicotinic acetylcholine receptor. Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. In mammals, the primary effects following acute high-dose oral exposure to imidacloprid are mortality, transient cholinergic effects (dizziness, apathy, locomotor effects, labored breathing) and transient growth retardation. It acts as an agonist at the postsynaptic nicotinic acetylcholine receptor (nAChR) in insects (Tomizawa Casida *et al*., 2005). Exposures to high doses may be associated with degenerative changes in the testes, thymus, bone marrow and pancreas. Cardiovascular and hematological effects have also been observed at higher doses. Animal studies are important because, in some instances, they have shed light on mechanisms of teratogenicity and because when such an agent causes similar patterns of anomalies in several species, human teratogens should also be suspected. Akhtar *et al*., 2006 studied on exposure to various environmental chemicals especially pesticides during developmental period is liable to give rise to congenital defects. One recent study by Capowiez *et al.,* 2006 presents very interesting data. The study was about the effect of neonicotinoids on the behavior of two earthworm specie. Epidemiological studies have shown neurobehavioral and cognitive deficits and increased susceptibility to disease in offspring at various developmental stages, all associated with maternal exposure to neurotoxic chemicals during pregnancy (Jacobson and Jacobson 2002). P. E. Natekar *et al*., 2012 observed malformations in Methotrexate treated group of chick embryo were stunted growth, break deformities, limb deformities, scanty feathers, short wings and ectopia vescerale. In 90 days oral toxicity study with imidacloprid in female rats at the concentration of 20 mg/kg/day evidenced decreased activity of acetyl choline esterase (AchE) in brain, spontaneous locomotor activity, histopathologically cerebellum of brain showed degenerative changes in purkinji cells and loss of

granules in granular layer (Bhardwaj *et al*., 2010). Recently imidacloprid has raised concern because of reports of egg shell thinning; reduced egg production and hatching time which are considered as signs of possible endocrine disruption (Berny *et al*., 1999 and Matsuda *et al*., 2001). A specific concern about imidacloprid is that it may cause similar developmental defects as the known teratogen nicotine. For developmental studies, chicken embryos are a model organism because they are inexpensive, easy to control with dosing, sensitive to toxins, and are vertebrates (Ejaz and Woong 2006). Increased use of chemical pesticides has resulted in contamination of the environment and many associated long-term effects on human health, ranging from short-term impacts such as headaches and nausea to chronic impacts such as cancer, reproductive harm, and endocrine disruption (Chen C *et al*., 2011). In view of the large-scale use of imidacloprid and the scarcity of Indian literature (Arora S. 2009). It is essential to assess the present environmental load of imidacloprid residues in different food commodities because imidacloprid is a toxic chemical (Kapoor U. *et al*., 2010; Tomizawa M. *et al*., 2003). The markets of industrialized countries for pesticides are no longer growing as their governments are putting restrictions or limiting the use of pesticides due to their serious health implications to man and his environment (Pretty J. *et al*., 2005; Savithri Y. *et al*., 2010).

Conclusion

In the light of current study, it can be concluded that the Neonicotinoid Insecticide imidacloprid is a potential teratogenic compound and therefore its use should be limited. Results shows that experimental group had comparatively more cases of growth retardation resulting into failure of retraction of yolk sac, head enlargement, scanty feathers, limbs deformities, beak deformities and ectopia viscerale as compared to the controls. Comparatively higher doses proved more toxic and also caused many morphological changes and developmental defects on chick embryos. India being agrarian state needs to take specific steps to educate farmers about pesticides ill effects and their judicious use so to limits its hazardous effect to the non-target species that are exposed to it directly or indirectly as taken along with food etc being residue in the agricultural products.

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