Scholars Journal of Applied Medical Sciences (SJAMS) ISSN 2320-6691 (Online)
Sch. J. App. Med. Sci., 2014; 2(1A):67-72 ISSN 2347-954X (Print)

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2014.v02i01.0014

Research Article

Malformations and Teratogenic Effects of Imidacloprid on Chick Embryo

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Abstract: Imidacloprid is one of the most commonly used insecticides worldwide belonging to the family of neonicotinoids and relatively new, systemic insecticide chemically related to the tobacco toxin nicotine. Like nicotine, it acts on nervous system. Worldwide, it is considered to be one of the insecticides used in the largest volume. It has a wide diversity of uses in agriculture, on turf, on pets, and for household pests. The present study was carried out in department of Anatomy Govt. Medical College, Ambedkar Nagar and Santosh Medical College Ghaziabad U.P. on 240 fertile eggs of white leghorn chicken obtained from government poultry farm after taking permission from animal ethical committee. Chicken eggs after having been exposed to Imidacloprid with doses of 10µg, 15µg, 25µg, and 40µg in a volume of 10µl, 15µl, 25µl and 40µl respectively and control same as test group. The embryos were terminated on 20th and 21st days, egg shell broken with a scalpel and embryos removed. Gross morphological abnormality observed and recorded in all embryos. The results show that experimental group had comparatively more cases of delayed and growth retardation resulting into failure of retraction of yolk sac as compared to the controls. Comparatively higher doses proved more toxic and also caused many developmental defects. Imidacloprid exposure increases the risks of developmental defects with increasing embryonic age. Imidacloprid caused developmental delays and malformations on chick embryo.
Keywords: Imidacloprid, chick embryo, malformations and teratogenic effects.

INTRODUCTION

Imidacloprid represents the new generation of neurotoxic insecticides, which exhibit more selective toxicity for insects relative to mammals. Since being introduced in the insecticide market in 1992, the use of imidacloprid has increased yearly. It ranked as one of the top selling pesticides in the world in 2001-2002. Imidacloprid is a relatively new class of neonicotinoid pesticide with a distinct mode of action [1]. Since it is a systemic chloronicotinyl insecticide that blocks the microtinergic neuronal pathway, it is used for control of sucking insects such as rice hoppers, aphids, ticks, white flies, termites, and turf insects. It is commonly used on rice, soya beans, maize, potatoes, cotton, sugar beets, and kitchen garden vegetables and fruits [2]. 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 [3]. Imidacloprid interacts with the acetylcholine receptor, which is widely conserved across species [4].

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 a consequence of this, the environmental monitoring and their impact assessment have become the priority areas of research. In Jan. 2013, the European Food Safety stated that neonicotinoids pose an unacceptably high risk to bees, and that the industry-sponsored science upon which regulatory agencies claims of safety have relied may be flawed, concluding that, A high acute risk to honey bees was identified from exposure via dust drift for the seed treatment uses in maize, oilseed ripe and cereals [5]. Pesticides are major contaminants of our environment and many persist in the environment including in various feeds and foodstuffs.

Global pesticide use is increasing, particularly in third world countries. India uses approximately 85,000 tons of pesticides per annum and an 8% increase in

pesticide use is expected every year. 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 reported by Kagabu [6]. In the Indian market, imidacloprid is included in the trade products Gaucho, for seed treatment, and Confidor, for leaf and soil treatment. Its use as a replacement for other insecticides is increasing. Developmental neurotoxicity study revealed decreased body weights, reduced motor activity level and changes in dimensions of brain structures. 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. For obvious reasons no studies of teratogenicity are conducted during embryogenesis of humans.

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 240 fertile eggs of white leghorn chicken obtained from the government poultry farm after taking permission from animal ethical committee. Eggs from stock known to be nutritionally healthy as well as free from genetic defects 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 place immediately in standard electrical digital incubator 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 CO_2 or O_2.

Injection Method of Imidacloprid in chick embryos:

Eggs will be candled on 3rd day to discard unfertilized eggs prior to injection. Eggs were divided into four groups A, B, C & D. Each group has 30 eggs each [14]. Control same as test group, treated with same volume of normal saline, whereas test group A, B, C&D were exposed to Imidacloprid with doses of 10µg, 15µg, 25µg and 40 µg in a volume of 10µl, 15µl, 25µl and 40µl respectively on 3rd 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. The needle was wiped with a sterile gauze pad between each injection and hole of the shell was sealed with Candle melted wax.

Table 1: Shows Injection of Imidacloprid in chick embryos in different groups

Doses	Groups							
	A		B		C		D	
	Control	Test	Control	Test	Control	Test	Control	Test
Normal Saline	10µl		15 µl		25µl		40 µl	
Imidacloprid								
10 µg		10µl						
15 µg				15µl				
25 µg						25µl		
40 µg								40 µl

METHODOLOGY

After injection of drug, eggs were again incubated at 38° C temperature and before hatching eggs were broken to collect embryos for examination on 20th day of incubation. The embryos were terminated and eggs removed from the incubator on 20th and 21st days, the egg shell were broken with a scalpel and the embryos were removed. The number of live and dead embryos was noted. Gross skeletal malformation were carefully observed and recorded in all the embryos. The embryos decapitated and the heads were fixed in 10% formaldehyde for a period of 1 day through 14 days. The dissection of chick embryo head was done and brains removed from the skull. Parameters namely the size of the head and the hardness of the tissue was evaluated. The gross anomalies and teratogenic effects of imidacloprid on chick embryo were observed and photographs. In the present study we used linear regression and ANOVA statistical tests were run on SPSS software and used for analysis of all resulting data.

RESULTS

The chick embryo were examined for malformations and we observed Growth Retardation (figure 3, 4), Head Enlargement (figure 5), Twisted Limbs (figure 4), Short beak (figure 6), Ectopia Viscerale (figure 6) and Failure of Retraction of Yolk sac (figure 1, 2, 3, 4) shown in table 2. Imidacloprid caused developmental delays or smaller embryos. The effects of imidacloprid on growth retardation overall statistically significant for embryos at 25 µg and 40 µg

levels. Imidacloprid had a significant adverse effects on embryo 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 ($p>0.001$).

Table 2: Shows malformations observed on 20th and 21st day in chick embryo after injection of Imidacloprid

Sl. No.	Abnormalities	Groups				
		Control	A	B	C	D
1.	Growth Reterdation	0	6	7	12	16
2.	Head Enlargement	0	0	0	2	4
3.	Twisted Neck and Limbs	0	0	2	2	5
4.	Short beak	0	0	0	1	2
5.	Ectopia Viscerale	0	0	0	1	2
6.	Failure of Retraction of Yolk Sac	4	10	13	15	17

Table 3: Shows lethal effects and malformations induced by Imidacloprid in chick embryo

Doses	No. of fertile eggs used	No. of dead Embryoes	No. of Live Embryoes
Control (N.S.)			
(A) 10 µl	30	0	30
(B) 15 µl	30	1	29
(C) 25 µl	30	2	28
(D) 40 µl	30	2	28
Imidacloprid			
(A) 10 µl	30	6	24
(B) 15 µl	30	7	23
(C) 25 µl	30	9	21
(D) 40 µl	30	12	18

Fig. 1: Shows Imidacloprid exposed chick with failure of retraction of yolksac (YS) in "A" Test group (T) and Control (C)

Fig. 2: Shows Imidacloprid exposed chick with failure of retraction of yolksac (YS) in roup "C" Test group (T) and Control (C)

Fig. 3: Shows Imidacloprid exposed chick with failure of retraction of yolksac (YS) in roup "B" Test group (T) and Control (C)

Fig. 4: Shows Imidacloprid exposed chick with failure of retraction of yolksac (YS) in roup "D" Test group (T) and Control (C)

Fig. 5: Shows Imidacloprid exposed chick with Head enlargement (HE) and growth retardation in "D" Test group (T) and Control (C)

Fig. 5: Shows Imidacloprid exposed chick with growh retardation, Ectopia Viscerale (EV) and twisted limbs

The lethal effects and malformations induced by Imidacloprid in chick embryo we found dead embryos in test A group 6(20%), B group 7(23.3%), C group 9(30%) and D group 12(40%) table 3. In control we observed in A Group all chick embryos were live, B Group 1(3.3%), C Group 2(6.6%) and D Group 2(6.6%) embryos found dead shown in table 3. The mortality rate was 4.1% in control group and 28.3% in test group, this difference was statistically significant ($p>0.05$).

DISCUSSION

The study of congenital anomalies is known as teratology. If an organ or organism clearly oversteps the reasonable limits in any range of variation, then the condition is known as abnormality, anomaly or malformation. 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.* studied on exposure to various environmental chemicals especially pesticides during developmental period is liable to give rise to congenital defects [7]. Administration of pregnant rats with a single extraperitoneal injection of imidacloprid at the rate of 337 mg/kg b. wt produced neurobehavioral deficits, increased expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats reported by Abou-Donia *et al.* [8].

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 studied by Bhardwaj *et al.* [9].

Administration of imidacloprid at the rate of 80 mg/kg b.wt/day through oral gavage for 28 days resulted in neuro toxicity which was evident from histopathological changes in brain like 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 reported by Soujanya *et al.* [10].

One recent study by Capowiez *et al.* presents very interesting data. The study was about the effect of neonicotinoids on the behavior of two earthworm specie [11]. P. E. Natekar *et al.* observed malformations in Methotrexate treated group of chick embryo were stunted growth, break deformities, limb deformities, scanty feathers, short wings and ectopia vescerale [12].

Recent findings suggest that thiamethoxam binds, compared to the other neonicotinoids sales products, in a different way, possibly to a different site of the receptor in aphids studied by Wellmann H *et al.* [13]. Knowledge of the most hazardous substances would enable medical professionals and would-be mothers to minimize foetal exposure to them, helping to achieve the laudable goal of abolishing teratogen-induced malformations.

CONCLUSION

Imidacloprid exposure increases the risks of malformations and teratogenic effects. In the light of present study, it can be concluded that the imidacloprid is a potential teratogenic compound and therefore its use should be limited. Results show that experimental group had comparatively more cases of growth retardation resulting into failure of retraction of yolk sac, head enlargement and ectopia viscerale as compared to the controls. Comparatively higher doses proved more toxic and also caused many developmental defects on chick embryo.

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