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Original Article

DEVELOPMENTAL AND BIOCHEMICAL EFFECTS OF IMIDACLOPRID ON CHICK EMBRYOS

Muktyaz Hussein*, Vishram Singh**, Prerna Gupta***, Birendra Yadav****, A.K.Singh*

*Department of Anatomy, Govt. Medical College Ambedkar Nagar.
**Department of Anatomy, Santosh Medical College Ghaziabad
***Department of Anatomy, Integral Institute of Medical Sciences and Research, Lucknow
****Department of Physiology, Govt. Medical College Ambedkar Nagar

ABSTRACT

Introduction: Environmental pollution is a worldwide problem in a modern society. 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 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.

Methods: Present study was carried out in the department of Anatomy Govt. Medical College, Ambedkar Nagar and Santosh Medical College Ghaziabad U.P. on 270 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 12.5µg, 25µg, and 50µg in a volume of 12.5µl, 25µl and 50µl respectively and control same as test group. The embryos were terminated on 21st day, egg shell broken with a scalpel and embryos removed. Biochemical and developmental changes observed and recorded.

Results: The results show that experimental group had comparatively more cases of developmental changes, growth retardation resulting into failure of retraction of yolk sac, limbs defects, and Ectopia Viscerale and biochemical changes as compared to controls.

Conclusion: Imidacloprid exposure increases the risks of developmental defects and biochemical parameters alterations with increasing embryonic age. Comparatively higher doses proved more toxic and also caused many developmental defects in chick embryos.

Keywords: Imidacloprid, chick embryos, developmental and biochemical effects.

INTRODUCTION

Imidacloprid (IMI) is one of the major representatives of the new generation of neonicotinoid insecticide have outstanding potency and systemic action for crop protection against piercing-sucking pests, and they are highly effective for flea control. It is one of the fastest sold insecticides across the world because of its high selective toxicity in insects and apparent safety in humans. 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 reported by Kagabu.[1] Imidacloprid interacts with the acetylcholine receptor, which is widely conserved across species.[2]

Environmental Pollution, when considered in its broadest context, is a by-product of human activities and its significance is in what ways it affects directly or indirectly the living population. 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. 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. 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.[3] The current study was designed to study the developmental and biochemical effects of imidacloprid on chick embryos.

Address for Correspondence:
Dr Muktyaz Hussein
Assistant Professor
Department of Anatomy, Govt. Medical College Ambedkar Nagar, U.P. India. Email: muktyazmukky@gmail.com
Contact No: 9161431601

MATERIALS AND METHODS

The present study was designed to assess the effect of Imidacloprid on chick embryos in the department of Anatomy Govt. Medical College, Ambedkar Nagar and Santosh Medical College Ghaziabad U.P. on 270 fertile eggs of white leghorn chicken weighing between 40 to 50 gm 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 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 Injection of Imidacloprid in chick embryos on 2nd day:

Eggs will be candled on 2nd day to discard unfertilized eggs prior to injection. Eggs were divided into three groups A, B and C. Each group has 45 eggs each. Control same as test group treated with same volume of normal saline, whereas test group A, B and C were exposed to Imidacloprid with doses of 12.5µg, 25µg and 50 µg in a volume of 12.5µl, 25µl and 50µl respectively 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. 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 and the hardness of the tissue were measured.

The embryos to be secrified and blood collected from the heart. Blood sample collected into a clot promoting vaccutainers and allowed to clot for 3 to 4 hours later centrifuged at 20k rpm for 10 minutes, serum was separated into tubes. Biochemical parameter namely blood urea, Serum creatinine, Serum total protein, and Serum Albumin and Serum uric acid done in biochemistry lab using standard methods using semi-automatic biochemical analyzer by employing standard biochemical kits. The dissection of chick embryo was done to observe the developmental changes and skeletal anomalies were carefully observed and photographs. Obtained data were analyzed statically.

RESULTS

The chick embryo were examined for developmental abnormalities and we observed Failure of Retraction of Yolk sac (figure 2, 3,4 and 6), Growth Retardation (figure 2, 3,5 and 6), Limbs deformities (figure 4), Head Enlargement (figure 5), Ectopia Viscerale (figure 6) and Scanty feathers (figure 5) shown in table 1.

TABLE 1. Shows developmental abnormalties on 21st day in chick embryos after injection of Imidacloprid.

S.NO	ABNORMALTIES	GROUPS			
		Control	A	B	C
1.	Growth Retardation	0	5	7	17
2.	Head Enlargement	0	0	1	3
3.	Limbs deformities	0	1	1	5
4.	Short beak	0	0	1	2
5.	Scanty feathers	0	1	2	4
6.	Ectopia Viscerale	0	0	2	3
7.	Failure of Retraction of Yolk sac	3	09	12	18

Imidacloprid caused developmental delays or smaller embryos. The effects of imidacloprid on growth retardation overall statistically significant for embryos at 25µg and 50µg levels. Imidacloprid had a significant adverse effects on embryo failure of retraction of yolk

TABLE 2. Shows lethal effects induced by Imidacloprid injection in chick embryos.

Doses	No. of fertile Eggs used	No. of dead Embryos	No. of Live Embryos
Control (N.S.) (A) 12.5µl	45	0	45
(B) 25µl	45	2	43
(C) 50µl	45	3	42
Imidacloprid (A) 12.5µl	45	8	37
(B) 25µl	45	10	35
(C) 50µl	45	13	32

sac although the control group has also shown the failure of retraction of yolk sac but the difference is statistically significant (p>0.001).

The lethal effects and malformations induced by Imidacloprid in chick embryo we found dead embryos in test A group 8(17.7%), B group 10(22.2%) and C group 13(28.8%) shown in table 2. In control we observed in A group all chick embryo were live, B group 2(4.4%) and C group 3(6.6%) embryos found dead shown in table 2. The mortality rate was 3.7% in control group and 22.9% in test group, this difference was statistically significant (p>0.05).

Photograph:1 shows Imidacloprid exposed chick embryos with developmental abnormalities failure of retraction of yolk sac (fig.2, 3, 5), growth retardation (fig.2, 3, 5and 6), Limbs deformities (fig. 4), Head Enlargement (fig.5), Ectopia Viscerale (fig.6) and Scanty feathers (fig.5) and control (C) is normal (fig.1).

Fig.1 Control (C)

Fig. 2 Group A (T)

Fig.3 Group B(T)

Fig.4 Group B (T)

Fig.5 group C (T)

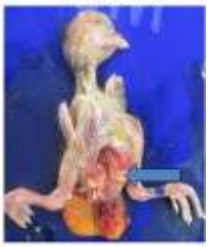
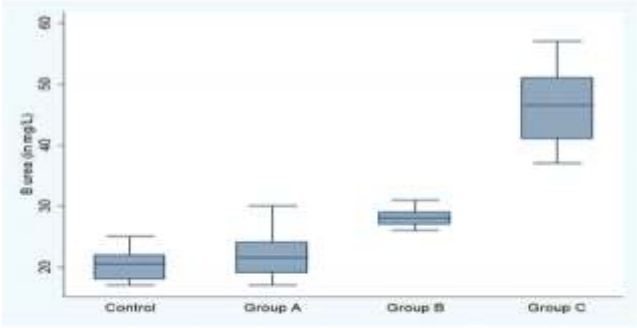


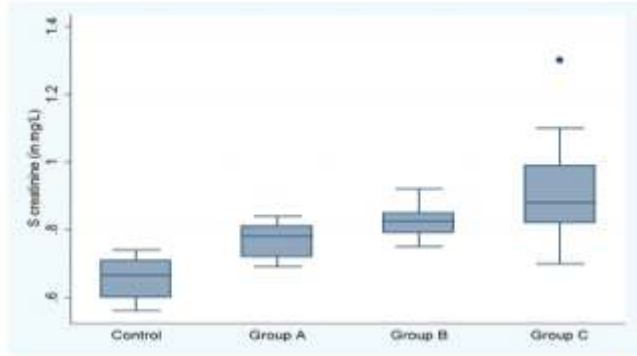
Fig.6 Group C(T)

The present biochemical study was conducted to evaluate the serum biochemical alterations induced by imidacloprid in chick embryos. The biochemical analysis showed a significant (P< 0.001) increase in blood urea in groups B and C; and serum creatinine in group B in comparison to control. Serum AST was significantly higher (p<0.001) in groups A, B and C; and serum ALT was also significantly higher (p<0.001) in group B and C in comparison to control. The biochemical effects of Imidacloprid on chick embryos serum parameter, namely blood urea, S.creatinine, S.total protein, S.Albumin, AST and ALT are shown in table no.3 to 8 and the corresponding box plots.



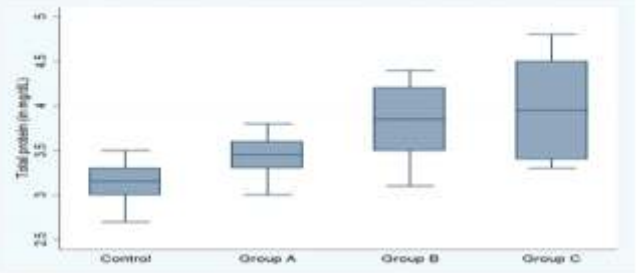
	Mean (in mg/L)	SD	P-value (compared to control group)
Control	20.30	2.63	-
Group A	22.10	4.07	P=0.255
Group B	28.00	1.49	P<0.001
Group C	46.50	6.80	P<0.001

Table.3 Shows effects of Imidacloprid on Blood Urea in chick embryos.



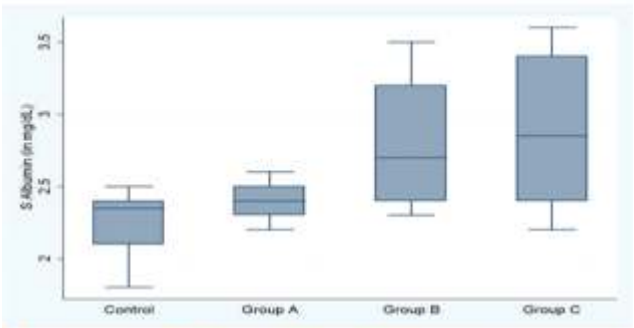
	Mean (in mg/L)	SD	P-value (compared to control group)
Control	0.66	0.06	-
Group A	0.77	0.05	P=0.0003
Group B	0.82	0.05	P<0.001
Group C	0.93	0.17	P=0.0002

Table.4 Shows effects of Imidacloprid on S. Creatinine in chick embryos.



	Mean (in mg/dL)	SD	P-value (compared to control group)
Control	3.14	0.25	-
Group A	3.43	0.25	P=0.018
Group B	3.84	0.41	P=0.0002
Group C	3.97	0.55	P=0.0004

Table.5 Shows effects of Imidacloprid on Total Protein in chick embryos.

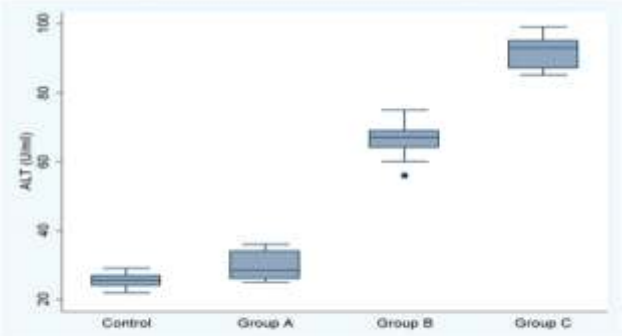


	Mean (in mg/dl)	SD	P-value (compared to control group)
Control	2.26	0.23	-
Group A	2.38	0.13	P=0.1718
Group B	2.81	0.47	P=0.0041
Group C	2.88	0.56	P=0.0048

Table.6 Shows effects of Imidacloprid on S. Albumin in chick embryos.

	Mean (in U/ml)	SD	P-value (compared to control group)
Control	20.50	1.90	-
Group A	30.50	5.50	P<0.001
Group B	62.6	6.29	P<0.001
Group C	97.60	11.63	P<0.001

Table.7 Shows effects of Imidacloprid on AST in chick embryos.



	Mean (in U/ml)	SD	P-value (compared to control group)
Control	25.60	2.07	-
Group A	29.60	4.09	P=0.0128
Group B	66.40	5.48	P<0.001
Group C	91.60	4.79	P<0.001

Table.8 Shows effects of Imidacloprid on ALT in chick embryos.

DISCUSSION

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. 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 (2005).[4] 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. studied on exposure to various environmental chemicals especially pesticides during developmental period is liable to give rise to congenital defects.[5] 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 species.[6] 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 & Jacobson (2002).[7] 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.[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] 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)[10] and Matsuda et al., (2001).[11]

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 reported by Ejaz and Woong (2006).[12] 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.[13] In view of the large-scale use of imidacloprid and the scarcity of Indian literature,[14] it is essential to assess the present environmental load of imidacloprid residues in different food commodities because imidacloprid is a toxic chemical.[15,16]

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. Balani et al. (2011) evaluated the toxic effects of oral administration of imidacloprid in male white leg horn chicken at 1.25, 1.67 and 2.5mg/kg b. wt for 28 days and noticed an increase in ALT, no change in AST, serum total protein, total globulins, total albumin and serum creatinine levels.[17] The increased levels of ALT and AST could be due to degeneration and necrosis of hepatocytes, which attributes an increased permeability of cell membrane that results in release of transaminases into the blood stream. H. kaur Toor, et al., (2013) reported that Imidacloprid induced biochemical alterations in liver also found in female albino rats.[18]

Chronic exposure to imidacloprid also induces inflammation and oxidative stress in the liver and central nervous system of rats (V.Duzguner, S. Erdogan, 2012).[19] Hepatotoxicity and nephrotoxicity were studied in layer chickens following single acute doses of 55 mg/kg bw and 139 mg/kg bw of CPF and Imidacloprid, respectively. The cholinergic signs in CPF intoxicated chickens were in agreement with those reported in chickens intoxicated with CPF and other organophosphate insecticides (Lumeij, 1997)[20] and Mohammad, (2007).[21] However, there is less information about the mechanism of the action of imidacloprid in chickens. Increase in serum creatinine levels might be due to failure of glomerular filtration process induced by imidacloprid toxicity to kidney. Decreased total protein levels were in accordance with observations of (Siddiqui et al., 2007)[22] and it may be attributed to imidacloprid induced hepato toxicity which was also evident from elevated serum ALT and AST activities.

CONCLUSION

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 embryos.

Biochemical analysis can provide valuable information for monitoring the health conditions of chick embryos. Biochemical analyses in the current study revealed that exposure to imidacloprid in chick embryo resulted in significantly higher values of blood urea ($P< 0.001$) at higher doses (Group B & C), serum creatinine ($P< 0.001$) in Group B, serum AST significantly higher ($p<0.001$) at all doses (Groups A, B, C) and serum AST ($p<0.001$) at higher doses (Groups B & C). The current study concludes that that exposure to Imidacloprid is associated with Hepatotoxicity and Nephrotoxicity in chick embryos.

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