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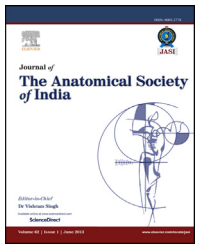

Original Article

Study on embryonic effects of neonicotinoid insecticide on chick embryos

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ABSTRACT

Introduction: Neonicotinoids are a group of 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. 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.

Methods: Present study was carried out in the Department of Anatomy Government Medical College, Ambedkar Nagar and Santosh Medical College Ghaziabad U.P. on 280 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 5 μg, 12.5 μg, 25 μg, and 50 μg in a volume of 5 μl, 12.5 μl, 25 μl and 50 μl respectively and control same as test group. The embryos were terminated on 18th and 20th days, egg shell broken with a scalpel and embryos removed. Gross abnormalities observed and recorded in all embryos.

Results: The results show that experimental group had comparatively more cases of delayed and growth retardation resulting into failure of retraction of yolk sac, limbs defects, neural tube defects as compared to controls. Comparatively higher doses proved more toxic and also caused many developmental defects.

Discussion: Neonicotinoid exposure increases the risks of developmental defects with increasing embryonic age. Imidacloprid caused developmental delays and defects on nervous system.

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1. Introduction

Neonicotinoids are widely applied pesticides due to their higher affinity for insect nicotinic acetylcholine receptors. These compounds are extensively applied to control pest insects in different agricultural crops; however they can also affect non-target organisms (humans or biota). Still a limited number of studies are referring to neonicotinoids in terms of potential hazard for the additive/cumulative effects on human health and to toxic effects of their transformation products on aquatic non-target organisms. The usage of high amount of pesticides in environment represents a possible risk for biota and human health due to their potential toxic action. 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 and plants) or to the environment.

The term 'neonicotinoid pesticides' comprises a group of several different insecticides, but it is usually used to indicate the four that are most widely applied imidacloprid, thiacloprid, clothianidin and thiamethoxam. One of these a representative, imidacloprid (IMI), was the main focus of this research. 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 (DNT) revealed decreased body weights, reduced motor activity level and changes in dimensions of brain structures (reduction in the thickness of corpus callosum and a decreased width of caudate putamen). 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.

2. 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 280 fertile eggs of white leghorn chicken (Cochran WG. Sampling Techniques)[7] 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 (Macro Scientific Pvt. Ltd.) with their broad end up where the chorioallantoic membrane is situated and were rotated three times daily along their longitudinal and vertical axis as advised by Olsen and Byerly.[8] 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.

2.1. *Method for injection of neonicotinoid (Imidacloprid) in chick embryos on 3rd day*

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 35 eggs each.[9] 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 5 μg, 12.5 μg, 25 μg and 50 μg in a volume of 5 μl, 12.5 μl, 25 μl and 50 μl respectively on 3rd day of incubation shown in Table 1.

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 egg shell 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