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Effects of Neonicotinoid Insecticide Imidacloprid on Hematological Parameters in Chick Embryos

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ABSTRACT

In order to meet the growing food demand, use of pesticides is a common feature in agriculture now a day but extensive use of pesticide has resulted in global contamination of the environment. Imidacloprid is one of the major representatives of the new generation of neonicotinoid insecticides. The objective of current study is to examine the hematological parameters and developmental defects after exposure of imidacloprid in chick embryos. Present study was carried out on 300 fertile eggs of white leghorn chicken. Imidacloprid exposure increases the risks of developmental defects and mild toxic effects on hematological parameters in chick embryos.

Introduction

The discovery of neonicotinoids as important novel pesticides can be considered a milestone in insecticide research of the past three decades. Modern agriculture depends on wide variety of synthetically produced chemicals including insecticides, fungicides, herbicides and other pesticides reported by Zhang et al., 2011 [1]. Neonicotinoids are a class of neuro active insecticides chemically related to nicotine. Neonicotinoids are insecticidal compounds produced by the tobacco plant. Their unique interaction with the insect nicotinic acetylcholine binding site establishes them as useful compounds for dealing with insect pests. 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. As a consequence of this, the environmental monitoring and their impact assessment have become the priority areas of research.

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 is possibly the most widely used insecticide, both within the mode of action group and in the worldwide market. It is now applied against soil, seed, timber and animal pests as well as foliar treatments for crops including: cereals, cotton, grain, legumes, potatoes, pome fruits, rice, turf and vegetables.

It is systemic with particularly effective against sucking insects and has a long residual activity. Imidacloprid is one such newer insecticide and is the first of the chemical class of neonicotinoids to be developed for commercial use. The application rates for neonicotinoid insecticides are much lower than older, traditionally used insecticides. Indiscriminate usage of pesticides in agriculture is leading to contamination of environment and natural resources, and thereby producing an adverse impact on animal and human health. A case of acute poisoning was reported in human following ingestion of a pesticide formulation containing 10% imidacloprid studied by Wu et al., 2001 [2] and two fatal intoxication cases have been reported recently by Proenca et al., 2005 [3]. Since imidacloprid is now being considered as a replacement for other existing pesticides, therefore the relative risk and benefits of this insecticide must be compared to the existing pesticides. Although an extensive literature exists on the basic hematology (e.g. hematocrit (PCV), red blood cell concentration (RBC) and blood hemoglobin concentration (Hb) of developing chicken embryos. The knowledge of embryonic hematological development in birds is thus somewhat fragmented and in need of integrated simultaneous measurement across ontogeny. The aims and objectives of our current study to examined the hematological parameters and developmental defects after exposure of imidacloprid insecticide in chick embryos.

Materials and Methods

The present current study was conducted in the department of Anatomy Govt. Medical College, Ambedkar Nagar and Santosh Medical College Ghaziabad U.P. to observe the effects of Neonicotinoid insecticides Imidacloprid on hematological parameters in chick embryos on 300 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 (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

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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 of 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 three groups A, B and C. Each group has 50 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 5µg, 10µg and 20 µg in a volume of 5µl, 10µl and 20µl 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. 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 and the numbers of live and dead embryos were noted. The gross developmental defects namely growth retardation, failure of retraction of yolk sac, limbs defects, head enlargement, scanty feather, beak defects and ectopia viscerale were carefully observed and photograph.

The embryos to be secrified and blood collected from the heart. Blood sample collected in EDTA vials for estimating hematological parameters namely hemoglobin (Hb), total leukocyte count (TLC) total erythrocyte count (RBC) and packed cell volume (PCV) using standard kits by hematology Analyzer (Medonic Sweden) and cell counter. The dissection of chick embryo was done to observe the others internal developmental changes and skeletal anomalies were observed and photographs. Obtained data were analyzed statistically.

Results

The present current study conducted to estimating hematological parameters namely hemoglobin (Hb), total leukocyte count (TLC), total erythrocyte count (RBC) and) packed cell volume (PCV) after exposure of imidacloprid in chick embryos. Our study showing significantly decrease levels of Hb ($P<0.001$) in all three groups compared to control group (figure 1), PCV was significant in group B and group C ($P<0.001$) compared to control group (figure 4). The present study did not show any significant changes in the levels of TLC and RBC (figure 2 &3) in all three groups compared to control group showing in table 1.

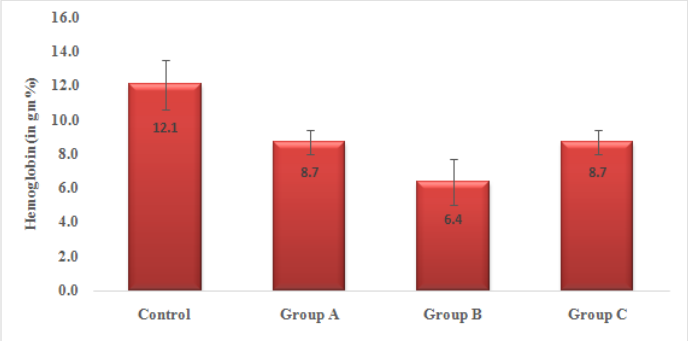


Figure 1. Shows effects of Imidacloprid exposure on Haemoglobin (Hb) in chick embryos.

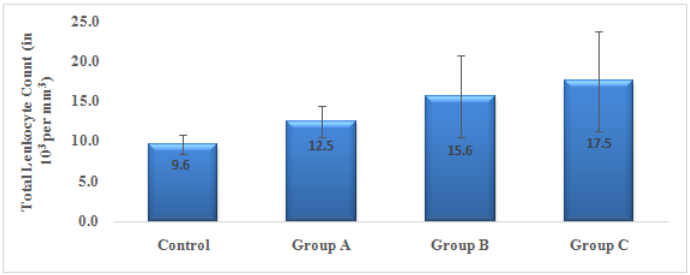


Figure 2. Shows effects of Imidacloprid exposure on Total Leukocyte Count (TLC) in chick embryos.

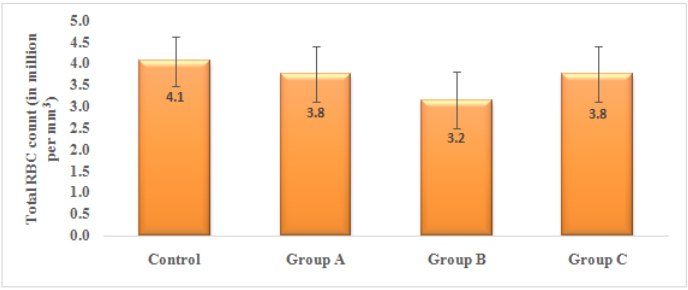


Figure 3. Shows effects of Imidacloprid exposure on Total Erythrocyte count (RBC) in chick embryos.

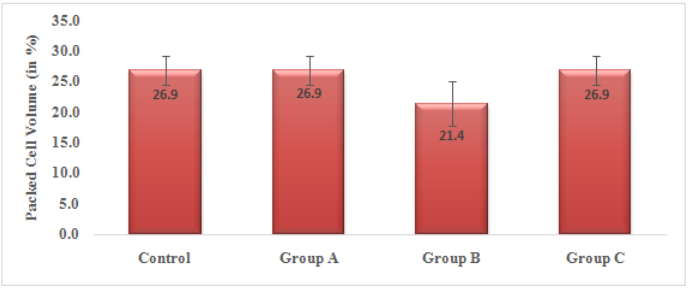
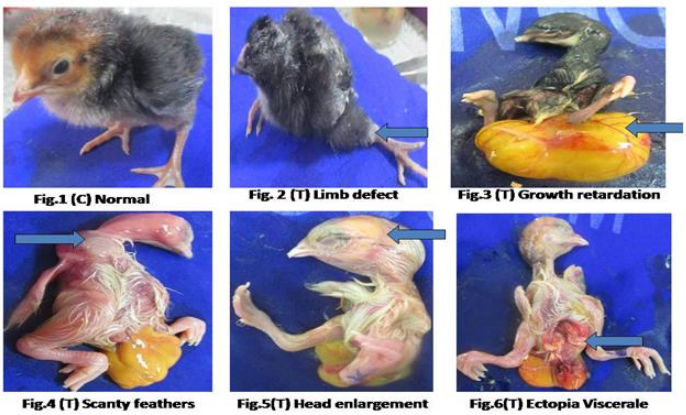


Figure 4. Shows effects of Imidacloprid exposure on Packed Cell Volume (PCV) in chick embryos.

In the present current study we observed gross developmental defects namely growth retardation (fig. 3, 5 and 6), failure of retraction of yolk sac (fig.3, 4, 5 and 6), limbs defects (fig.2), scanty feathers (fig.4), head enlargement (fig.5), beak defects and ectopia viscerale (fig.6) compared to control group (fig.1) shown in table 2 and photograph 1.



Photograph 1 shows developmental defects after exposure to Imidacloprid in chick embryos; figure 1 control (C) normal, figure 2 Test (T) limb defects, figure 3 Test (T) Growth retardation, figure 4 Test (T) Scanty feathers, figure 5 Test (T) head enlargement and figure 6 Test (T) ectopia viscerale.

Discussion

Indiscriminate usage of pesticides in agriculture is leading to contamination of environment and natural resources, and thereby producing an adverse impact on animal and human health.

Table 1. Shows effects of Imidacloprid exposure on hematological parameters in chick embryos

Groups	Hb (in gm %)			TLC (in 10^3 per $mm^{3)}$			RBC(in million per mm^3			PCV (in %)		
	Mean	SD	*P- value	Mean	SD	*P -value	Mean	SD	*P- value	Mean	SD	*P- value
Control	12.07	1.43	-	9.64	1.18	-	4.06	0.58	-	26.9	2.38	-
Group A	8.67	0.71	P<0.001	12.49	2.00	P=0.001	3.76	0.64	P=0.286	26.9	2.38	P=1.00
Group B	6.37	1.34	P<0.001	15.6	5.09	P=0.002	3.15	0.66	P=0.004	21.4	3.63	P<0.001
Group C	8.67	0.71	P<0.001	17.52	6.21	P=0.001	3.76	0.64	P=0.286	26.9	2.38	P<0.001

* P-value compared to control group.

Imidacloprid, a neonicotinoid insecticide, is extensively used in agriculture for control of the sucking insects and coleopteran beetles reported by Cox, 2001 [4] and also used as foliar treatment for soil and for seed dressing by Felsot, 2001 [5]. Ammar et al., 2003 [6] reported a significant increase in the total leukocytes count due to administration of imidacloprid at 0.1 and 0.25 LD50 dose in male albino rats.

Bhardwaj et al. 2010 [7] documented an insignificant change in hematological parameters (RBC, WBC, Hb, HCT, MCV and DLC) in female rats by oral administration of imidacloprid at the rate of 5, 10, 20 mg/kg b. wt for 90 days in their experiment. Recently imidacloprid has raised concern because of its ability to cause egg shell thinning, reduced egg production and hatching time, which are considered as signs of possible endocrine disrupters studied by Matsuda et al.,2001 [8]. The knowledge of embryonic hematological development in birds is thus somewhat fragmented and in need of integrated simultaneous measurement across ontogeny Oxygen demand increases during embryonic development, requiring an increase in red blood cells (RBCs) containing hemoglobin (Hb) to transport O2 between the respiratory organ and systemic tissues.

A hematological analysis is routinely used in determining the physiological state of animals which is known to be affected by different environmental factors and is used as a guide in the diagnosis of many diseases in both animals and humans studied by Solomon and Okomoda, 2012 [9]. In order to meet the growing food demand, use of pesticides is a common feature in agriculture now a days but extensive use of pesticide has resulted in global contamination of the environment and only 0.1 % of the applied pesticides reach the pests and remaining 99.9 % find their way to different components of environment reported by Marigoudar et al., 2009 [10]. Ammar et al., 2003 reported a significant increase in the total leukocytes count due to administration of imidacloprid at 0.1 and 0.25 LD50 dose in male albino rats [6]. Sridhar 2010 also reported an insignificant change in hematological parameters (Hb, PCV, TEC, TLC and DLC) in natural cases of imidacloprid toxicity in buffaloes [11]. Soujanya et al. 2012 evaluated that oral administration of imidacloprid at the rate of 80 mg/kg b. wt for 28 days in male albino rats produced a significant decrease in TEC, Hb, PCV, MCV, MCH and MCHC and a significant increase in TLC. Whereas co-administration of vitamin-C brought mild to moderate improvement in all these parameters [12]. Balani et al. 2011 conducted an experiment in male white leg horn chicks for 28 days by administering imidacloprid at the rate of 1.25, 1.67, 2.5 mg/kg b. wt and observed an insignificant change in haematological parameters (Hb, PCV and TEC) except decreased TLC counts at 2.5 mg/kg body weight [13]. A few cases of significant human toxicity due to imidacloprid have been reported in medical literature. In a prospective human case series of 68 cases, the majority of the cases developed mild gastrointestinal symptoms and only one case required mechanical ventilation for respiratory failure [14].

Conclusion

In the light of current study, it can be concluded that the imidacloprid is a potential teratogenic compound and therefore its use should be limited. 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.

Imidacloprid has shown mild toxic effects on hematological parameters in chick embryos. Results show that experimental group had comparatively more cases of growth retardation resulting into failure of retraction of yolk sac, limb defects, scanty feathers, head enlargement and ectopia viscerale as compared to the controls. Comparatively higher doses proved more toxic and also caused many developmental defects in chick embryos.

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