Mortality and congenital defects after injection of various doses of Imidacloprid in chick embryos with the comparison of normal saline

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ABSTRACT:
Introduction: The Imidacloprid are widely used in agriculture to curb the loss caused by insects. The Imidacloprid are incorporated into the food chain and accumulate in the human body, as well interrupt the various metabolic pathways. The Imidacloprid is a widely applied pesticide due to their higher affinity for insect nicotinic acetylcholine receptors, it acts on nervous system. The insecticides exposure can be linked to cancer, endocrine disruption, reproductive effects, neurotoxicity, kidney and liver damage, birth defects, and developmental changes in a wide range of species.

Aim: To observe mortality in chick embryos after exposure to various doses of Insecticide Imidacloprid in comparison to control.

Methods: This present study was carried out in the department of Anatomy Govt. Medical College, Budaun and Government medical college Ambedkar Nagar U.P. 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 5µg, 10µg, 20µg and 40µg in a volume of 5µl, 10µl, 20µl and 40µl respectively and control same as test group.
The embryos were terminated on 20th day, egg shell broken with a scalpel and embryos removed. The mortality in chick embryos observed and recorded.

Results: The results show that experimental group had comparatively more cases of mortality in chick embryos in compared to controls.

Conclusion: Imidacloprid exposure increases the mortality and developmental defects with increasing embryonic age. The comparatively in our study higher doses proved more toxic and also caused many developmental defects.

Keywords: Mortality, normal saline, chick embryos, congenital defects.

INTRODUCTION:
The Imidacloprid are substances that exist in our day to day lives. The most widespread use of Imidacloprid in agriculture, where they are used to protect crops from pests caused by plants and animals. They are also useful to prevent diseases caused by ecto parasites in farm the animals and pets. The insecticides substances are used in gardening and brought into our homes to protect us from mosquitoes and other insects. The Imidacloprid come into intimate touch with all forms of life through the drinking water and eating food. However, the use of these substances is so widespread and poorly controlled that the environmental contamination is inevitable. The environmental pollution is a worldwide problem in a modern society and the extensive uses of pesticides are widely used to enhance the crop production and other benefits and have increased concerns about the potential harmful effects on the environment, human health and non-target animals.

The Imidacloprid 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-nicotinyl insecticide. It was the first member of a new family, the neonicotinoids, and is chemically related to the nicotinic acetylcholine receptor agonists nicotine and epibatidine. The insecticides Imidacloprid was identify 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. Imidacloprid interacts with the acetylcholine receptor, which is widely conserved across species. The Imidacloprid is a systemic chloronicotinyl insecticide and is used as an insecticide in agriculture, where pesticides are widely used to control pests and other organisms. The insecticides are also beneficial for the agricultural industry and are used to reduce the environmental impact of pest control.

In the past some years agricultural production has been enormously increased by the use of many synthetic pesticides. Although, their use is based on selective toxicity for certain organisms yet it has resulted in serious effects on many non-target organisms as well. The more application of pesticides has created a type of chemical environment which is proving the 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 the human or animal disease or weed which can cause the harm during or otherwise obstruct with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs.

MATERIALS AND METHODS:
This current study was carried out in the Anatomy department research lab Govt. Medical College, Budaun and Govt. Medical College, Ambedkar Nagar U.P. on 400 fertile eggs of white leghorn chicken weighing between 38 to 55 gm obtained from the government poultry farm after taking permission from the animal ethical committee. The eggs from stock known to be nutritionally healthy were taken. The 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 the standard 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 set at 60-80 percent with no additional CO2 or O2 and the eggs were tilted three to four times per day.

Method for Injection of doses Imidacloprid in chick embryos on the 2nd day:
Eggs will be candled on 2nd day to discard unfertilized eggs prior to injection. 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 Insecticides Imidacloprid with doses of 5µg, 10µg, 20µg and 40µg in a volume of 5µl, 10µl, 20µl and 40µl respectively on 2nd day of incubation shown in table1.

| TABLE 1: Shows doses of Imidacloprid in chick embryos in different groups. |
|-----------------|-----------|-----------|-----------|-----------|-----------|
| DOSES           | GROUPS    | A         | B         | C         | D         |
| Normal Saline   | Control   | Test      | Control   | Test      | Control   |
| Imidacloprid    | 5µl       | 10µl      | 20µl      | 40µl      |

Implication: The results show that experimental group had comparatively more cases of mortality in chick embryos in compared to controls. Imidacloprid is a systemic chloronicotinyl insecticide and is used as an insecticide in agriculture, where pesticides are widely used to control pests and other organisms. The insecticides are also beneficial for the agricultural industry and are used to reduce the environmental impact of pest control.
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 prevent 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 20th day, the egg shell were broken with a scalpel and the embryos were removed. The number of live and dead embryos was recorded and mortality in chick embryos after exposure to varying concentrations of Insecticide Imidacloprid in comparison to controls groups were noted and observed carefully.

RESULTS:
In our study we found number of live chick embryos in test group A, B, C and D were 38,35,31,26 respectively and dead chick embryos observed in test group A, B, C and D were 12,15,19,24 respectively. We found more dead embryos in group C (38%) and D (48%) statistically highly significant (p>0.001). The live and dead chick embryos after exposure to varying concentrations of Insecticide Imidacloprid in comparison to controls groups showing in table 2.

Table 2: Showing live and dead chick embryos after exposure to varying concentrations of Insecticide Imidacloprid in comparison to controls groups

<table>
<thead>
<tr>
<th>Doses</th>
<th>No. of fertile eggs used</th>
<th>No. of live chick embryos</th>
<th>No. of dead chick embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline) Group A (5µl)</td>
<td>50</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>Group B (10µl)</td>
<td>50</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>Group C (20µl)</td>
<td>50</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>Group D (40µl)</td>
<td>50</td>
<td>46</td>
<td>4</td>
</tr>
<tr>
<td>Test (Imidacloprid) Group A (5µl)</td>
<td>50</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>Group B (10µl)</td>
<td>50</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Group C (20µl)</td>
<td>50</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>Group D (40µl)</td>
<td>50</td>
<td>26</td>
<td>24</td>
</tr>
</tbody>
</table>

In our study on mortality in chick embryos exposed to varying concentrations of Insecticide Imidacloprid in comparison to controls group showing total number of dead chick embryos in test group A (5 µl) were 12 (24%), control group A (5 µl) 2 (4%), test group B (10µl) were 15 (30%), control group B (10µl) 2 (4%), test group C (20µl) were 19 (38%), control group C (20µl) 5 (10%). The highest mortality in chick embryos after exposure found in test group D (40µl) were 24 (48%), control group D (40µl) 4 (8%) showing in table number 3.

Table: 3 Showing the mortality in chick embryos exposed to varying concentrations of Insecticide Imidacloprid in comparison to controls groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Total number of chick embryos</th>
<th>Total number of dead chick embryos (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>5 µl Imidacloprid</td>
<td>50</td>
<td>12 (24%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Control</td>
<td>5 µl normal saline</td>
<td>50</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>GROUP B</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Test</td>
<td>10 µl Imidacloprid</td>
<td>50</td>
<td>15 (30%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>10 µl normal saline</td>
<td>50</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>GROUP C</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Test</td>
<td>20 µl Imidacloprid</td>
<td>50</td>
<td>19 (38%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>20 µl normal saline</td>
<td>50</td>
<td>5 (10%)</td>
<td></td>
</tr>
<tr>
<td>GROUP D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Imidacloprid had a significant mortality on chick embryos although the control group has also shown mortality but the difference is statistically significant (p<0.001).

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 quadrupedal animals, the primary effects following acute high-dose oral exposure to insecticides imidacloprid are mortality, transient cholinergic effects (dizziness, apathy, locomotor effects, labored breathing) and transient growth retardation.

Exposures to high doses may be related with the degenerative changes in the testes, thymus, bone marrow and pancreas. Cardiovascular and hematological effects have also been observed at higher doses. The 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.

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. The recent study on 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 (Bermey et al., 1999) and Matsuda et al., (2001). A definitive concern about imidacloprid is that it may cause similar embryological 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). The Increased use of pesticides has resulted in contamination of the environment and many related long-term effects on the human health, ranging from short-term impacts such as headaches and nausea to chronic impacts such as cancer, reproductive harm, and endocrine disruption. In view of the large-scale use of imidacloprid and the scarcity of Indian literature, it is essential to assess the present environmental load of imidacloprid residues in different food commodities because the insecticide imidacloprid is a toxic chemical substance.

CONCLUSION:

In the light of our present study, it can be concluded that the insecticide Imidacloprid is a potential teratogenic compound and therefore its use should be decline. This study results shows that test groups had comparatively more mortality and congenital defects than normal saline. Comparatively higher doses proved more harmful and also caused high mortality on chick embryos.

REFERENCES:


<table>
<thead>
<tr>
<th>Test</th>
<th>40 µl Imidacloprid</th>
<th>50</th>
<th>24 (48%)</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40 µl normal saline</td>
<td>50</td>
<td>4 (8%)</td>
<td></td>
</tr>
</tbody>
</table>


