RESEARCH ARTICLE

Evaluation of Imidacloprid Induced Changes in Thyroid Gland and its Amelioration with Withania somnifera in Wistar Rats

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INTRODUCTION

Neonicotinoids are the most widely used newer class of insecticides due to its lower mammalian toxicity and highly selective insecticidal activity. These are broad spectrum and systemic insecticides with rapid action. Imidacloprid (IMI) is the first registered neonicotinoid insecticide used to control insect pests on agricultural crops and ectoparasitic arthropods on animals. It has been introduced into the insecticide market in 1992, since then the use of IMI has been increased progressively and ranked as one of the top selling pesticides in the world during 2001-2002 (Swenson and Casida, 2013). It accounts for about 25 % of the current global insecticide market (Swenson and Casida, 2013). It acts as a potent agonist on insect nicotinic acetylcholine receptors (nAChRs), specifically at the α-subunits of the nicotinic receptors (Matsuda et al., 2005). Several lab animal studies revealed the IMI as an endocrine disruptor (Kapoor et al., 2011; Abbassy et al., 2014; Nabiuni et al., 2015; Hafez et al., 2016).

Withania somnifera (WS) is a potential medicinal Indian herb used in Ayurveda for more than 3000 years. WS belongs to Solanaceae family, commonly known as ashwagandha, Indian ginseng and winter cherry (Girdhari and Rana, 2007). The plant extract has many bioactive compounds and thereby exerts antioxidant, immunomodulatory, anti-aging, anticancer, anti-inflammatory and anti-stress activities. Studies in mice have shown that the WS root extract has thyrotropic effect, involved in increasing the thyroxine (T4) hormone levels and thus capable of stimulating the thyroid function (Panda and Kar, 1999). In view of this, the current research work was designed to evaluate the toxicity of IMI on thyroid gland of female Wistar rats and its amelioration by Withania somnifera.

MATERIALS AND METHODS

The experiment was conducted according to the guidelines and with prior approval of the Institutional Animal Ethics Committee (IAEC-No.7/22/C.V.Sc., Hyd./IAEC).
**Experimental Animals**

In the present study forty-eight (48) female albino rats (Wistar strain) weighing 200-250 gm were procured from Sanzyme Private Limited, Gagan Phahad, Hyderabad. The rats were housed in solid bottom polypropylene cages at lab animal house, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana (India) and were maintained in controlled environment (Temperature 20-22°C) throughout the course of the experiment. 3 rats were housed per cage and maintained for one week for acclimatization prior to treatment. Sterile rice husk was used as a bedding material. All the rats were provided with standard pellet diet (procured from Vyas Labs, Uppal, Hyderabad) and deionized water *ad libitum* throughout the experimental period. All the experimental animals were observed thrice daily for clinical signs and mortality, if any, during the entire period of study.

**Experimental Design**

A total of 48 female Wistar rats were divided into 4 groups each containing 12 rats. Group 1 was control, group 2 was orally given with imidacloprid at the rate of 30 mg/kg body weight/day, group 3 was treated with *Withania somnifera* at the rate of 1 gm/kg feed and group 4 was administered with both imidacloprid and *Withania somnifera* (dose as above) for a period of 30 days. The imidacloprid dose was calculated as three times to that of NOEL (Kapoor et al., 2010).

**Blood Collection and Hormone Estimation**

Blood samples were collected from six rats in each group on 16th day and from remaining six rats on 31st day of experiment. From each rat, approximately 2 mL of blood was collected from retro orbital plexus through capillary tube into a clot promoting vacutainers and allowed to clot for 3 to 4 hours, later centrifuged at 2000 rpm for 10 min, serum was separated into eppendorf tubes and stored at -20°C. The serum samples were analyzed for thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) by using enzyme linked immunosorbent assay (ELISA) kits (Krishgen Bio-systems, Mumbai).

**Necropsy Observations**

Six rats from each group were sacrificed on 16th day and remaining were sacrificed on 31st day of the experiment by cervical dislocation a day after collection of blood and detailed postmortem examination was carried out as per standard procedure suggested by Feinstein (2000).

**Relative Organ Weight**

Soon after sacrifice, individual weight of thyroid gland from all the rats were recorded by using electronic balance to study the relative organ weight. Relative organ weight was expressed as per cent (%) of body weight in relation to body weight. Individual body weights of all the rats were recorded by using electronic balance.

**Histopathology and Ultra-Structural Pathology:**

The thyroid gland tissue samples were collected and fixed in 10 % neutral buffered formalin (NBF) soon after necropsy. The samples were processed, sectioned (5 μm) and stained with Hematoxylin and Eosin (H&E) for histopathological examination as per the standard procedure in practice. The samples of thyroid gland were collected and preserved in 2.5 % gluteraldehyde (PBS based EM grade) and processed for scanning electron microscopy (SEM) as per the standard protocol of Ruska Labs, Hyderabad (Lakshman, 2019). Later specimens were observed under SEM (JEOL; JSM-5600, Japan).

The data obtained were subjected to statistical analysis by one way ANOVA using statistical package for social sciences (SPSS) version 20.0. Differences between the means were tested by using Duncan’s multiple 40 comparison test and significance level was set at p<0.05 (Snedecor and Cochran, 1994).

**RESULTS AND DISCUSSION**

**Thyroid Stimulating Hormone - TSH (μIU/mL):**

The mean values of TSH (μIU/mL) were significantly (p<0.05) higher in group 2 and group 4 when compared with group 1 and group 3 on 16th and 31st day of experiment. The mean value of TSH was significantly (p<0.05) lower in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 on 16th and 31st day of experiment (Table 1). Similarly increase in TSH levels due to imidacloprid administration were documented by Pandey and Mohanty (2017).

TSH is secreted by thyrotrhops of the anterior pituitary gland in response to thyroid-releasing hormone (TRH) from the hypothalamus and it stimulates the thyroid follicular cells to synthesize and release T3 and T4 into circulation. In the present study, the increased TSH level in group 2 rats was suggestive of disturbance in hypothalamic pituitary thyroid axis. Hypothetically, the IMI might have induced negative feedback of TSH on the anterior pituitary resulted by low levels of T3 and T4 in circulation. Hence, the IMI is also responsible for thyrotoxicity. The elevated TSH levels in group 2 rats were supposed to stimulate the thyroid follicular cells to increase the synthesis and release of T3 and T4, but the thyroid follicular cells were again under the influence of IMI, this cycle might have continued and resulted in persistent reduction of T3, T4 and elevation in TSH levels.

**Triiodothyronine - T3 (ng/mL):**

Significantly (p<0.05) decreased mean values of T3 (ng/mL) were recorded in group 2 when compared with group 1 on 16th and 31st day of experiment. There was no significant difference in mean values of T3 between groups 1, 3 and 4 on 16th and 31st day of experiment, respectively (Table 1).
**Thyroxine - T4 (μg/dL):**
The mean values of T4 (μg/dL) in groups 2 and 4 were significantly (p<0.05) decreased when compared with groups 1 and 3 on 16th and 31st day of experiment. In group 4, there was a significant (p<0.05) increase in T4 values in comparison to group 2, and group 3 value was insignificant from control (Table 1). The decrease in T4 levels due to imidacloprid administration was in agreement with earlier workers (Abbassy et al., 2014; Ibrahim et al., 2015; Kamel and Cherif, 2017; Pandey and Mohanty, 2017). Contrary to this, Saadi et al. (2014) reported a non-significant decrease in the levels of T3 and T4 in female rats treated with IMI.

Thyroid gland is the only source for synthesis of T3 and T4 in animals. The thyroid follicular cells release 80% of T4 and 20% of T3 into circulation in response to TSH and then the T4 get converted to T3 through de-iodination process in circulation (Pirahanchi et al., 2020). The decreased levels of T3 and T4 in current experiment might be due to reduced conversion of T4 in to T3 in circulation and this could also be due to the decreased synthesis of T3/T4 by degenerated thyroid follicular epithelial cells. The degeneration of thyroid follicle cells might be due to oxidative damage induced by IMI. The histopathology and ultra-structural pathology of thyroid gland in group 2 rats strongly supporting the serum biochemistry (decreased levels of T3 and T4). It also might be due to antagonistic effect of IMI on thyroid receptors as described by Ibrahim et al. (2015). IMI may compete with T3 for binding to thyroid receptors and result in disruption of thyroid receptor function (Bhaskar and Mohanty, 2014).

**Relative Thyroid Gland Weight (% of Body Weight)**
No significant difference was observed in mean values of relative thyroid weights (% of body weight) between groups 1, 2, 3 and 4 on 16th and 31st day of experiment, (Table 1). Similarly, no significant change in relative thyroid weight was earlier recorded by Vohra et al. (2014).

**Histopathology**
The sections of thyroid gland of group 2 rats on 16th day of experiment revealed moderate congestion of inter-follicular blood vessels, degenerated thyroid follicles with loss of colloid substance, increase in inter-follicular space, atropic follicles and mild fibrosis (Fig. 1a, 1b, 1c). Some other sections showed acini filled with vacuolated and granular colloid (Fig. 1d). On 31st day, disrupted follicles, severe congestion of inter-follicular blood vessels, shrunken follicles filled with vacuolated and granular colloid, coalesce of follicles forming bullae, pyknotic nuclei and detachment of follicular epithelial cells from basement membrane into lumen (Fig. 1e, 1f, 1g, 1h) were observed.

**Table 1:** Mean (±SE) values of thyroid stimulating hormone (TSH), Triiodothyronine (T3) Thyroxine (T4) and Relative thyroid gland weight (% body weight) in different groups of rats on day 16 and 31 post-treatment (n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>TSH (μIU/mL)</th>
<th>T3 (ng/mL)</th>
<th>T4 (μg/dL)</th>
<th>Thyroid weight (% of B.Wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>0.43±0.03c</td>
<td>0.47±0.03c</td>
<td>1.98±0.09a</td>
<td>2.02±0.13a</td>
</tr>
<tr>
<td>Group-2</td>
<td>1.16±0.08a</td>
<td>1.28±0.09a</td>
<td>1.02±0.11b</td>
<td>0.95±0.14b</td>
</tr>
<tr>
<td>Group-3</td>
<td>0.51±0.03c</td>
<td>0.54±0.04c</td>
<td>1.82±0.27a</td>
<td>2.03±0.22a</td>
</tr>
<tr>
<td>Group-4</td>
<td>0.84±0.04b</td>
<td>0.94±0.05b</td>
<td>1.67±0.25a</td>
<td>1.65±0.25b</td>
</tr>
</tbody>
</table>

Means with different superscripts within the column differ significantly at p<0.05.
Other sections showed increase in number of micro-follicles and dilated inter-follicular area (Fig. 2a). Some sections of thyroid gland (group 2) revealed moderate fibrous tissue proliferation in inter-follicular area, degenerated follicles with loss of colloid and proliferation of follicular epithelium towards lumen (Fig. 2b, 2c). The thyroid gland sections of group 4 rats showed mild vacuolation of colloid, mild congestion of inter follicular blood vessels and increase in number of micro follicles (Fig. 2d, 2e) on 16th day of experiment. Similar lesions were also noticed on 31st day of experiment. In addition, coalesce of follicles forming bullae, increased inter-follicular space and degeneration of follicles (Fig. 2f, 2g, 2h) were noticed on 31st day.

Histopathological lesions induced by IMI in thyroid gland in present experiment were similar with the earlier studies i.e., the increase in microfollicles number and loss of colloid in thyroid gland sections due to imidacloprid treatment were reported by Saadi et al. (2014) in rats. Disruption of follicles and altered colloid volume in thyroid gland sections due to imidacloprid administration was recorded by Pandey and Mohanty (2015) in birds. These changes suggest the inflammation in thyroid gland, which could be due to oxidative damage caused by generation of free radicles by IMI. Comparatively minimal lesions were observed in thyroid gland sections of group 4 rats than group 2 rats. It might be due to antioxidant and thyrotropic nature of WS. Similarly, the improvement in histoarchitecture of thyroid gland due to administration of WS was reported by Abdel-Wahhab et al. (2019) in hypothyroidism modeled rats.

Ultra-structural Pathology

Scanning electron microscopic examination of thyroid gland of groups 1 and 3 rats showed normal architecture of thyroid follicles filled with granular colloid and connective tissue in-between the follicles (Fig. 3a, 3b, 3c, 3d). The thyroid gland of group 2 rats on 16th day of experiment revealed increase
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The Indian Journal of Veterinary Sciences and Biotechnology, Volume 19 Issue 5 (September-October 2023)

In number of micro-follicles, degenerated thyroid follicles with loss of colloid substance (Fig. 3e, 3f), haemorrhage and moderate fibrosis in inter-follicular area (Fig. 4a, 4b).

On 31st day, disrupted follicles and severe inter-follicular fibrosis (Fig. 4c, 4d) were observed. The thyroid gland of group 4 rats showed mild degeneration of thyroid follicles with loss of colloid substance on 16th day of experiment (Fig. 4e). Mild inter-follicular fibrosis was also noticed on 31st day (Fig. 4f).

**Fig. 4:** Scanning electron micrograph of thyroid gland showing: a) haemorrhage in inter-follicular area (Gp 2, Day 16). b) Moderate inter-follicular fibrosis (Gp 2, Day 16). c) Disrupted follicles (Gp 2, Day 31). d) Severe inter-follicular fibrosis (Gp 2, Day 31). e) Mild degeneration of thyroid follicles with loss of colloid substance (Gp 4, Day 16). f) Mild inter-follicular fibrosis (Gp 4, Day 31).

Ultrastructural changes in thyroid gland of group 2 rats could be due to the free radical damage induced by IMI. In group 4 rats, the thyroid gland showed mild lesions in comparison with group 2. It indicates the potential ability of the IMI to induce oxidative damage despite of WS supplementation.

**Conclusions**

It could be concluded from the study that exposure to imidacloprid (IMI) induced marked alterations in serum thyroid hormone levels suggestive of endocrine disruption. IMI also caused histopathological and ultra-structural changes in thyroid gland. It could be due to IMI induced oxidative stress that resulted in degeneration of follicular epithelial cells. The co-administration of *Withania somnifera* (WS) at the rate of 1 gm/kg feed along with the IMI revealed mild to moderate improvement in all the parameters, which indicated that WS provided moderate protection against the toxic effects of IMI. It might be due to thyrotropic nature of WS that resulted in regeneration of thyroid follicular epithelium.

**Acknowledgement**

We are thankful to the Dean, Faculty of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India for providing the facilities to carry out the research work.

**References**


ANNOUNCEMENT

X Annual Convention and National Symposium of SVSBT-2023

Extension of Date of Abstract Submission

This is to inform that on request from many participants, the last date of submission of Abstract through e-mail svsbt2023@gmail.com is extended till 23rd September, 2023 for presentation in the X Annual Convention of the Society for Veterinary Science & Biotechnology (SVSBT) and National Symposium on “Recent Biotechnological Advances in Health and Management of Livestock, Poultry and Companion Animals” to be Hosted by College of Veterinary Science & Animal Husbandry (NDVSU, Jabalpur), Mhow, Indore, M.P. during 5th to 7th October, 2023. The other details floated in Brochure cum Invitation remain unchanged. The abstracts received after 23rd September, 2023 will not be entertained.

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