EFFECT OF THIOUREA ON THE THYROID GLAND IN THE TADPOLES OF TOAD, *BUFO STOMATICUS*

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**ABSTRACT**

The present study deals with the effect of Thiourea on the tadpole larvae of *Bufo stomaticus*. These tadpole larvae identified into three stages-pre-metamorphic (stage 24-25), pro-metamorphic (stage 34-35), and metamorphic climax (stage 38-40). They were exposed to five different doses of Thio-urea i.e. 0.04%, 0.08%, 0.1%, 0.2% and 0.3% of the solution. Experiments were conducted at room temperature (28ºC-32ºC) for 15 days. Tadpoles larvae of each group were morphologically examined under stereoscopic binocular microscope to find out the changes in size of thyroid gland. The results were statistically analyzed and significant changes were observed in some groups.

**Key words:**- Tadpoles, Thio-urea, *Bufo stomaticus* and Thyroid gland.

**INTRODUCTION**

There are many concerns about endocrine disrupting chemical effects on wild animals as well as humans. Considerable numbers of chemical have been shown to alter the thyroid system in vertebrates and disruption of thyroid axis has been identified as an important consideration for the regulation of chemicals. Amphibian tadpoles are very sensitive to environmental substances because of their habitat and the complex process of metamorphosis regulated by the endocrine system mainly thyroid hormones i.e. thyroid gland. A number of agents are known which are anti thyroid in their action and can be used effectively in making the animals athyroid or hypothyroid, such agents when used in amphibians larvae, result in retarding, delaying or even completely inhibiting metamorphosis [17, 18, 19, 6, 2].
Hypothyroidism induced either by administration of an antithyroid drug, thiourea or by surgical thyroidectomy, inhibits or reduces ovulation and spawning in fishes and frogs\(^{[21,23,24]}\). In the anura prolactin was reported by some authors\(^{[1,22,7,8]}\) to provoke the blockage of metamorphosis. Prolactin was also observed to inhibit the stimulating effect of exogenous TSH (Thyroid Stimulating Hormone) but not to interfere with the action of exogenous thyroxine\(^{[10]}\). Thyroid gland produces two hormones, tetra-iodothyronine (thyroxine ;T4) and triiodothyronine (T3), these hormones have ubiquitous effect on tissue metabolism, differentiation and maturation in tetrapod\(^{[4,14]}\). Development, growth and activity of the gland itself effect under hypothalamic control. Thiourea suppress the thyroidal activity by influencing thyroid gland. It affect upon the development of thyroid gland. Thiourea has moderate chronic toxicity to aquatic life. The effect of thiourea concentration on copper electrodeposition was studied\(^{[9]}\). It is well known antithyroid agent and it is known to show complicated behavior in own its deposition\(^{[2]}\).

**MATERIAL AND METHODS**

(a) Collection of sample:– Todpoles of toad *Bufo stomaticus* were collected from water near Albert hall in Ram Niwas garden at Jaipur. They were brought to the laboratory in polythene bags and kept in small troughs.

(b) Experimental set up: - These tadpoles identified into three stages- pre-metamorphic stage (stage 24-25), pro- metamorphic stage (stage 34-35 ) and metamorphic climax (stage 38-40) developmental stages according to Khan(1965). Each stage divided into six groups of 50 tadpoles. Tadpoles of group-I were reared in water(Control ) while those of groups II –VI were reared in 0.04%, 0.08%, 0.1%, 0.2% and 0.3% solution of thiourea for 15 days. The experiments were carried out at room temperature (28ºC-32º C). The rearing medium was changed on alternate day and the tadpoles were fed on freshly half boiled spinach leaves.

(c) To study the changes:– The larvae of each group were narcotized in 1:4000 solution of MS\(^{222}\) (Sandoz) and examined under stereoscopic binocular microscope. Changes in thyroid glands were observed at different developmental stages pre, pro and metamorphic climax stages. Data was statistically analysed.

**RESULTS AND DISCUSSION**

It was observed that in Group-I(Control) percentage of survivality was 100% and no mortality was noticed, the animals were normal in all respects.
(a) Pre- metamorphic stage: Data represent mean size of thyroid gland which decreased upto 6.56µm in Group-VI. At this stage the S.D. was highest in Group-III and it was lowest in Group-II.

(b) Pro metamorphic stage: At this stage mean size and S.D. of thyroid gland which decreases 9.70 µm in Group-VI. At this stage standard deviation was high in Group-II and it was lowest in Group-V.

(c) Metamorphic climax: At this stage the size of thyroid gland was also decreased according to the increasing level of thiourea. Table- 1 represents mean size and S.D. of thyroid gland which decreased upto 16.28 µm in Group-VI (Table 1), (Fig-1.A,B,C).

The size of thyroid gland reflects the state of activity of the thyroid hormone. In the inactive state, it regress to a low squamous state. Some drugs inhibits the primary action of the formation of thyroid hormone, these compounds are known as antithyroid drugs. Antithyroid drugs inhibit the formation of thyroid hormone by interfering with the binding of iodine into an organic form. The changes occurring in the thyroid histological pattern and metabolism in amphibian larvae have been regarded as reflecting antithyroidal action of prolactin [25,11,12].

At pre- metamorphic and pro- metamorphic stage, the effect of thiourea is not significant because at this stage level of T3 and T4 is lower. But at metamorphic climax the plasma T4 and T3 concentration are high. Thiourea, it is also known as antithyroid drug, suppress the thyroid activity. Thyroid hormone sulfating activity to be present in liver cytosol in frog todpales and the T3:T4 sulfating activity ratio varies during developmental stages [20].

In lower doses 0.04% and 0.08% THU at metamorphic climax stage, animals were small than the control. At higher doses 0.1%, 0.2% and 0.3% THU head portion is very small against the body region and the size are also reduce. This results shows that thiourea suppress the thyroid gland and promote hypothyroidism condition. Thiourea decreases the secretion of T3 and T4 that influences metamorphosis in tadpoles. Deiodinase enzymes are found in several peripheral target tissues in amphibians and the level of expression is closely connected with metamorphosis [14,4]. Same result was found, influences of prolactin on thyroid activity to differ according to the larval stages [12,5,3].
FIG-1. Effect of fifteen days treatment with thio-urea on the tadpoles of toad, *Bufo stomaticus* at room temperature (28°C-32°C)

(A) Pre-metamorphic stage; (B) Pro-metamorphic stage; (C) Metamorphic climax
(B) Furthermore, evidences has been provided that prolactin, antithyroid drug induces the appearance of thyroxine dependent arginase activity in the liver of Bufo larvae at an earlier stage as compare to normal animals [13].

(C) Chemical interfering with T3 directly affect the free concentration of plasma T3 and its plasma clearance rate [26,15]. The metamorphosis processes are systematically controlled by TSH in Xenopus laevis [17]. Many scientist reported effects of exposure to PTU (propylthiouracil) and another peroxidase inhibitor ethylene thiourea in Xenopus laevis [18,19]. After exposures to PTU also known as inhibit deiodinase, Xenopus tropicalis tadpoles showed considerable reduction in the developmental stage [6]. Electrochemical behavior of thiourea and its activity was noticed by many scientist [9,20,2].

Table- 1. Size of Thyroid glands in tadpoles of toad, Bufo stomaticus treated with thiourea at room temperature (28°C-30° C)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rearing medium</th>
<th>Pre-metamorphic stage</th>
<th>Pro-metamorphic stage</th>
<th>Metamorphic climax</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ±S.E. (in µm)</td>
<td>S.D.</td>
<td>mean ±S.E. (in µm)</td>
</tr>
<tr>
<td>I</td>
<td>Control (water)</td>
<td>11.42±0.59</td>
<td>1.34</td>
<td>16.56±0.420</td>
</tr>
<tr>
<td>II</td>
<td>0.04% THU</td>
<td>10.98±0.432**</td>
<td>0.96</td>
<td>15.22±0.425**</td>
</tr>
<tr>
<td>III</td>
<td>0.08% THU</td>
<td>8.20±0.391</td>
<td>0.874</td>
<td>14.84±0.393</td>
</tr>
<tr>
<td>IV</td>
<td>0.1% THU</td>
<td>7.78±0.389</td>
<td>0.870</td>
<td>12.16±0.365</td>
</tr>
<tr>
<td>V</td>
<td>0.2% THU</td>
<td>6.72±0.312**</td>
<td>0.697</td>
<td>10.28±0.270**</td>
</tr>
<tr>
<td>VI</td>
<td>0.3% THU</td>
<td>6.56±0.247</td>
<td>0.554</td>
<td>9.70±0.311</td>
</tr>
</tbody>
</table>

Significance  ** P<0.01 Significant;  *** P<0.001 Highly Significant; THU- Thiourea

Table 2 : Level of T4 and T3 at different metamorphic stages in tadpoles of toad, Bufo stomaticus

<table>
<thead>
<tr>
<th>Rearing medium</th>
<th>Pre metamorphic stage</th>
<th>Pro metamorphic stage</th>
<th>Metamorphic climax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>T4 µg/g</td>
<td>T3 µg/g</td>
<td>T4 µg/g</td>
</tr>
<tr>
<td></td>
<td>0.88±0.51</td>
<td>0.80±0.56</td>
<td>1.97±0.42</td>
</tr>
<tr>
<td>0.04% THU</td>
<td>0.68±0.86</td>
<td>0.34±0.04</td>
<td>0.89±0.83</td>
</tr>
</tbody>
</table>
CONCLUSION

In this study data indicate that thio urea inhibit the development of thyroid gland and it suppress thyroidal activity i.e. T3 and T4 hormonal level in plasma serum, that affect on the metamorphosis in all stages of tadpole larvae mainly on metamorphic climax stage of *Bufo stamaticus*, at higher doses of thiourea, it inhibit thyroidal activity and suppress the metamorphosis.

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| 0.08% THU | 0.40±0.45 | 0.21±0.02 | 0.68±0.86 | 0.35±0.21 | 2.68±0.86 | 1.23±0.43 |
| 0.1% THU | 0.36±0.82 | 0.21±0.02 | 0.60±1.04 | 0.35±0.21 | 1.98±0.42 | 0.82±0.15 |
| 0.2% THU | 0.22±0.51 | 0.09±0.01 | 0.49±0.77 | 0.26±0.20 | 1.83±0.40 | 0.80±0.06 |
| 0.3% THU | 0.11±0.35 | 0.06±0.01 | 0.48±0.63 | 0.26±0.20 | 1.12±1.34 | 0.54±0.06 |


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