TOXICOPATHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF THYROID GLAND OF RAT IN FLUOROSIS

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ABSTRACT
The thyroid gland has strong capacity for absorbing and accumulating fluoride. The present study elucidates effect of fluoride on the structure and function of thyroid gland. Wistar albino rats were treated with sodium fluoride in the doses of 300 and 600 mg/kg bodyweight/day by oral gavage for 40 days. The controls were given 1ml double distilled water/kg body weight /day for the same period. The rats were provided *ad libitum* with tap water and fed with standard commercial rat chow. The animals were anaesthetized and thyroid glands were excised and processed for histopathological examination. The thyroid gland of control rat exhibited many lobules separated by trabeculae. The follicular walls were lined by a single uniform layer of flattened to cubical follicular cells, with oval to round nuclei and contained abundant densely colloid material within the lumen. Groups of inter follicular cells and blood capillaries were observed in the connective tissue between the follicles. In fluoride treated animals, the thyroid gland showed increase in the number of follicles. Most of the follicles were enlarged, fused and distended with vacuolated colloid. Some follicles have no colloid in their lumens. Micro follicles with narrow lumen were visible along with increasing inter follicular spaces. Inflammatory cells in the epithelial lining of the follicles were observed. Hyperatrophied follicles with an increase in the height of their lining epithelium were prominent. The thyroid gland exhibited necrosis with esinophilic rounded droplets in the cytoplasm of necrotic cells, congested blood vessels and decrease of colloid content. Serum concentration of T3 and T4 decreased significantly (P<0.05) in animals treated with 300 and 600 mg NaF as compare to control. The serum level of TSH was not statistically significant in control and fluoride treated rats.

Keywords: Pathology, Sodium Fluoride, Thyroid Gland, T3, T4, TSH

INTRODUCTION
Endemic fluorosis is related to the high concentration of fluoride present in the drinking water. It is a slow and progressive process that causes metabolic, functional and structural damages which have been reported in many tissues, particularly musculoskeletal, dental tissue, kidney, liver, brain and endocrine glands (Selim et al., 2012).

The thyroid gland is the most important endocrine gland for metabolic regulation. The thyroid hormones, thyroxin and triiodothyronin are necessary for the growth, metabolism and functioning of virtually every cell in the body (Sojka, 1995).

The gland is organized in to spherical follicles whose walls are composed of epithelial cells that surround a lumen filled with colloid.

Each follicle is lined by a single layer of cells. The size and shape of the follicles depends upon the activity of the thyroid gland (Parchami and Dehkordi, 2012).

Fluoride disturbs the synthesis and secretion of thyroid hormone interferes with the activity of enzyme that catalyze the conversion of thyroxin into the active thyroid hormone (Rehman and Fetouh, 2013, Shashi and Singla, 2013).

Increase dietary fluoride resulted in thyroid enlargement and caused structural and functional changes (Patil and Dhurvey, 2015). Fluoride effects several parameters of thyroid activity. However, its effect on histopathology has not been studied extensively. Therefore, this study aimed to examine the histopathological and functional changes in the thyroid gland of albino rats induced by chronic fluoride exposure.
MATERIALS AND METHODS
Young Wistar albino rats, weighing between 100-150 g were housed in polypropylene cages with stainless grill tops and fed with standard rat pellet diet and water was given ad libitum. The experiments were performed under the approval of the animal ethical committee of Punjabi university Patiala. (Animal maintenance and Registration No.107/99/CPCSEA/2014-37)

Experimental Design
Rats were allowed a 2- week acclimatization period and then they were divided randomly into three groups (6 rats each).
Group I: This group served as a control and received 1ml double distilled water/kg bw/day orally daily by a gastric tube for 40 days.
Group II: This group of rats received fluoride at a dose of 300 mg/kg bw/day for 40 days orally by gastric tube.
Group III: This group received fluoride at a dose of 600 mg/kg bw/day for 40 days.
The control and experimental animals were sacrificed under ether anaesthesia. The thyroid gland was dissected out, washed in normal saline and processed for histopathology examination.

Histopathological Study
The thyroid tissue was fixed in 10% formalin, dehydrated in ascending grades of alcohol, cleared in amyle acetate and embedded in Paraffin wax. Serial sections were cut at 7 μm and stained with haemotoxylin and eosin.

Assessment of Thyroid Hormones
The estimation of T3, T4 and TSH in serum samples of control and fluorotic animals was done by using commercially available enzyme immunoassay test kit (Bio check, Inc. California).

Statistical Analysis
The data were analyzed with sigma stat (SPSS, version 16 for windows Inc., Chicago, IL, USA). The mean values and standard deviations of all parameter were calculated. Statistical analysis was performed using ANOVA. The level of significance was set at P<0.05.

Pathological Lesions
Group I
The thyroid gland of control rat showed many lobules separated by trabeculae (Figure 1). The follicular walls were lined by a single uniform layer of flattened to cubical follicular cells, with oval to round nuclei and contained abundant densely colloid material within the lumen (Figure 2).

Figure 1: T.S. of Thyroid Gland of a Control Rat Showing Thyroid Follicles Lined by Follicular Epithelial Cells Containing Colloid; The Follicles are Variable in Size Forming the Gland Lobules Separated by Trabeculae (arrow); H&E x 200
The epithelial cell height was normal and no inter follicular spaces were apparent. Groups of inter follicular cells and blood capillaries were observed in the connective tissue between the follicles (Figure 3).

**Group II**

In animals treated with 300 mg NaF/kg bw/day, the thyroid gland showed increase in the number of follicles. Most of the follicles were enlarged, fused and distended with vacuolated colloid (Figure 4). Some follicles have no colloid in their lumens. Microfollicles with narrow lumen were visible (Figure 5) along with increasing inter follicular spaces (Figure 6). Inflammatory cells in the epithelial lining of the follicles were observed (Figure 7). Hyperatrophied follicles with an increase in the height of their lining epithelium were also seen (Figure 8).
Figure 4: T.S of the Thyroid Gland of Rat Treated with 300mg/kg bw/Day of Sodium Fluoride Showing that most of the Follicles are Enlarged, Fused (Arrow) and Distended with Vacuolated Colloids; H&E x 400

Figure 5: T.S of Thyroid Gland of Rat Treated with 300mg/kg bw/Day of Sodium Fluoride Showing Follicles with no Colloid in their Lumens. Other Follicles are Fused, with an Extensive Vacuolated Colloid; Microfollicle with Narrow Lumen are Visible; H&E x 400

Figure 6: T.S of Thyroid Gland of Rat Treated with 300mg/kg bw/Day of Sodium Fluoride Showing Increasing Interfollicular Spaces; H&E x 400
Figure 7: T.S of Thyroid Gland of Rat Treated with 300mg/kg bw/Day of Sodium Fluoride Showing Some Inflammatory Cells in the Epithelial Lining of the Follicles; H&E x 1000

Figure 8: T.S of Thyroid Gland of Rat Treated with 300mg/kg bw of Sodium Fluoride Showing an Hyper Atrophied Follicle (Arrow) with an Increase in the Height of their Lining Epithelium; the Lining Follicular Cells are Large, Cuboidal having Rounded Nuclei with an Extensive Vacuolated Cytoplasm; H&E x 1000

Group III
The thyroid gland of rat treated with 600mg/kg bw/day of sodium fluoride showed fusion of follicles with vacuolated scanty colloid in the colloidal lumina (Figure 9). Thyroid gland exhibited necrosis with esinophelic rounded droplet in the cytoplasm of necrotic cell (Figure 10, 11). Focal epithelial hypertrophy, cytoplasmic vacuoles and the thyroid follicles contained minimal amount of colloid (Figure 12). Necrosis of the epithelial follicular cells, lining of glandular acinia and severe hemorrhage (Figure 13) were observed. Congested blood vessels and overall necrosis of the follicular cells and decrease of colloid content was present. Microfollicle with single layer of flattened to cubical cells were visible (Figure 14).
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Figure 9: T.S of Thyroid Gland of Rat Treated with 600mg/kg bw/Day of Sodium Fluoride Showing Fusion of Follicles with Vacuolated Colloids; H&E x 200

Figure 10: T.S of the Thyroid Gland of Rat Treated with 600mg/kg bw/Day of Sodium Fluoride Showing Necrosis with Eosinophilic Rounded Droplet in the Cytoplasm of Necrotic Cell; H&E x 200

Figure 11: T.S of Thyroid Gland of Rat Treated with 600mg/kg bw/Day of Sodium Fluoride Showing Fused Follicles (Arrow); Follicles have Vacuolated Scanty Colloid in the Colloidal Lumina; H&E x 400
Figure 12: T.S of the Thyroid Gland of Rat Treated with 600mg/kg bw/Day of Sodium Fluoride Showing Focal Epithelial Hypertrrophy, Cytoplasmic Vacuoles and the Thyroid Follicles Contain Minimum Amount of Colloid; H&E x 1000

Figure 13: T.S of the Thyroid Gland of Rat Treated with 600mg/kg bw/Day of Sodium Fluoride Showing Necrosis of the Follicular Cell Lining of Glandular Acinia and Severe Hemorrhage; H&E x 1000

Effect of Fluoride on Levels of Thyroid Hormones
One way ANOVA (F=14.393, P<0.05) showed that serum concentration of T₃ decreased significantly in thyroid gland of animals treated with 300mg NaF, further in the animals treated with 600mg NaF, serum T₃ level showed more decline (F=29.800, P<0.05) as compared to control and 300mg NaF groups (Table 1; Figure 1)

Table: 1 Mean serum level of T₃ (ng/dl) in control and fluoride treated rat.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Dose (mg NaF/kg b.w)</th>
<th>n</th>
<th>T₃ (ng/dl) Mean ± SEM</th>
<th>% Age Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (Control)</td>
<td>1ml Double Distilled Water</td>
<td>6</td>
<td>59.20 ± 2.478</td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>300mg NaF</td>
<td>6</td>
<td>47.80 ± 3.153</td>
<td>-19.256</td>
</tr>
<tr>
<td>Group-III</td>
<td>600mg NaF</td>
<td>6</td>
<td>32.60 ± 1.503</td>
<td>-25.675</td>
</tr>
</tbody>
</table>

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Figure 14: T.S of the Thyroid Gland of Rat Treated with 600mg/kg bw/Day of Sodium Fluoride Showing Microfollicle (MF); These Follicles are Lined by Cuboidal Cells with Rounded Vesicular Nuclei and Vacuolated Cytoplasm; Fusion of Follicles is also Seen; H&E x 1000

![Image of thyroid gland](image)

Figure: Mean Serum Level of T3 in Animals of Control and Sodium Fluoride Treated Groups

One way ANOVA showed that serum concentration of T₄ decreased significantly (P<0.05) in thyroid gland of animals treated with 300 (F=61.533) and 600 mg sodium fluoride (F=96.200) as compared to control (Table 2; Figure 2).

Table: 2 Mean Serum Level of T₄(µg/dl) in Control and Fluoride Treated Rats

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Dose (mg NaF/kg bw)</th>
<th>n</th>
<th>T₄(µg/dl) Mean ± SEM</th>
<th>% Age Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (Control)</td>
<td>1ml Double Distilled Water</td>
<td>6</td>
<td>3.360 ± 0.262</td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>300mg NaF</td>
<td>6</td>
<td>3.220 ± 0.215</td>
<td>-4.166</td>
</tr>
<tr>
<td>Group-III</td>
<td>600mg NaF</td>
<td>6</td>
<td>2.280 ± 0.269</td>
<td>-32.142</td>
</tr>
</tbody>
</table>
The serum level of TSH in NaF treated rats decreased in group II and group III as compared to group I (Control), however the changes were not statistically significant (Table 3).

Table: 3 Mean Serum Level of TSH (µIU/ml) in Control and Fluoride Treated Rats

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Dose (mg NaF/kg bw)</th>
<th>n</th>
<th>TSH (µIU/ml) Mean ± SEM</th>
<th>% Age Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (Control)</td>
<td>1ml Double Distilled Water</td>
<td>6</td>
<td>3.424 ± 0.615</td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>300mg NaF</td>
<td>6</td>
<td>1.576 ± 0.119 NS</td>
<td>-53.97</td>
</tr>
<tr>
<td>Group-III</td>
<td>600mg NaF</td>
<td>6</td>
<td>1.200 ± 0.131 NS</td>
<td>-64.95</td>
</tr>
</tbody>
</table>

**Discussion**

The present study showed severe damage and destruction in thyroid gland tissue parenchyma, hemorrhage and vacuolation in cytoplasm of follicular cells which may be due to severe inflammation present in thyroid gland. It leads to vasodilatation and vasoconstriction which damage or congestion of blood vessels, causes oozing of blood and fluid edema with infiltration of mono nuclear cells. This evidence was consistence with Liu *et al.*, (2008) who predicted that fluoride causes morphological structure in thyroid tissue of rats, after administered 50, 100 and 200 mg/L of NaF in drinking water, for 150 days. Jwad (2014) also reported hemorrhage in capsular region of thyroid gland, neutrophils infiltration in lumen, as well as edema marked vacuolation of cytoplasm of colloid cell, and granulomatous lesion seated in gland parenchyma.

The study demonstrated that the rats treated with NaF showed that the follicular cells marked decrease in cell height in addition to reduced colloid content. The decreased height reflects the decreased activity of the follicular cells. The result agrees with the study of Liu *et al.*, (2002) who suggested that fluoride can induce structural changes and dysfunction in the thyroid gland.

The decreased colloid content observed in the present work is in agreement with the study of Bouaziz *et al.*, (2005) who observed a decrease in the colloid content in mice treated with NaF in addition to increased follicular number and vascularity. The degenerative changes of the follicular epithelial cells examined can be explained by Barbier *et al.*, (2010) who suggested that, fluoride interacts with a wide range of cellular processes, such as gene expression, cell cycle, proliferation and migration, respiration, metabolism, ion transport, secretion, apoptosis and oxidative stress. Patil and Dhurvey (2015) found that in rats treated with NaF in the concentration of 5 to 20 ml/kg bw/ through drinking water for 15 days,
thyroid gland showed decrease in colloid volume, increase in inter follicular spaces and fusion of the follicles. In the present study, high fluoride water significantly decrease the level of the serum T₃ and T₄. Consistent with our finding, decreased T₃ and T₄ levels were also observed by Mc Laren (1976) who stated that the thyroid function may be influenced by the fluorine intake higher than 5mg/day. Hara (1980) found that 1, 5, 10, 50, 100 and 200 ppm fluoride in water could decrease the serum T₃ and T₄. Yu (1985) reported a decrease serum T₄ level and increase TSH level in the residents of endemic fluorosis area. Jwad (2014) reported decreased level of T₃ and T₄ with increase of TSH in rat treated with 150 and 500 mg/kg bw of NaF for 60 days. Despite the effects on thyroid hormones observed in our study, we did not see a statistical effects on the level of TSH. A previous study suggests that fluoride can inhibit the activity of thyroid adenylate cyclase, which suppresses the stimulating effect of TSH in the process. Therefore, it is possible that fluoride may affect the activity of TSH without detectable effects on the TSH serum level.

Declaration of Interest
The author reports no conflicts of interest.

REFERENCES
Jwad BM (2014). Acute and chronic pathological effects with biochemical alteration in thyroid gland induced by NaF in wistar rats. AL-Qadisiya Journal of Veterinary Medical Sciences 13(1) 66-74.