# Effects of Soy Isoflavones and Tamoxifen on The Structure of Thyroid Gland of Adult Female Versus Male Albino Rats

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# Conclusion

The present work clarified that soy isoflavones and tamoxifen have estrogen agonistic effects that was gender related. Male thyroid tissue in both groups was more affected than the female ones with more deleterious effects on male rats receiving soy isoflavones. Thus, it is advisable that soy based foods should be limited in order to control their undesirable stimulatory effects on human thyroid that might lead to hypothyroidism, thyroid autoimmunity or even neoplasm formation. On the other hand, cancer patients receiving medications containing soy isoflavones or tamoxifen should have a routine follow up for their thyroid profile to avoid any undesirable thyroid effects.

# Summary

The present work investigated the effects of soy isoflavones, a naturally occurring selective estrogen receptor modulator (SERM), and tamoxifen, a synthetic SERM, on the structure of thyroid gland of both male and female adult albino rats.

Sixty adult albino rats, thirty males and thirty females, were used in this study. Animals were divided into three groups each containing ten males and ten females. Rats in group I were given the vehicles of the drugs and were used as control. Animals in group II were given soy isoflavones at a dose of 500µgm/gm for three months and those in group III were given tamoxifen at a dose of 200 µg /kg tamoxifen once per day for four weeks. At the end of the experiment, animals were sacrificed; thyroid glands were excised, processed and examined using light and electron microscopes. Morphometric studies were also done.

Our results showed that male rats given soy isoflavones exhibited assorted follicles, localized hyperplasia and honeycomb appearance of the colloid. Semithin sections showed markedly dilated blood vessels and many mast cells. In addition, electron microscopic examination revealed that the follicular cells had irregular nuclei, extensively dilated rER and multiple lysosomes.

Parafollicular cells showed euochromatic nuclei and their characteristic secretory granules with variable electron densities.

Thyroid gland of female rats given soy isoflavones showed marked hyperplasia where the follicles were lined with multiple layers of follicular cells and the hyperplasic cells were also seen extending from one side of the follicle or as isolated clusters between the follicles. Numerous highly congested blood vessels were also encountered. Semithin sections also revealed that follicles were lined by double or multiple layers of follicular cells and showed groups of cells with indistinct cell boundaries placed in between the follicles. Mast cells were frequently detected and some of them were seen degranulated releasing their contents.

Electron microscopic examination clarified that the follicular cells contained slightly irregular nuclei, markedly dilated rER, numerous lysosomes and some secretory vesicles. Well circumscribed groups of cells were also observed between the follicles. Parafollicular cells with their secretory granules of variable densities were also encountered.

Morphometric studies of the soy isoflavones group revealed highly significant increase in the surface area and perimeter of follicles in both males and females.

Regarding male rats given tamoxifen, their thyroid glands showed distorted architecture. Follicles were lined by flattened

epithelium and were distended with colloid having marked peripheral vacuolation. Aggregations of hyperplasic cells and congested blood vessels were also encountered. Semithin sections revealed extensive wavy fibers separating the follicles or extending to surround individual ones.

Ultrastructural examination revealed mildly dilated rER and dense lysosomes scattered throughout the cytoplasm. Parafollicular cells with their characteristic granules were also seen.

Thyroid glands of female rats given tamoxifen showed marked flattening of the follicular epithelium and peripheral vacuolation of the colloid distending the follicles. Multiple congested blood vessels were also frequently observed. Semithin sections revealed marked colloid distention with subsequent flattening of follicular epithelium. Fibrous tissue surrounding aggregated follicles was also seen in this group.

Ultrastructural examination showed flattened follicular cells with elongated nuclei, basal vesicles and numerous apical microvilli. Groups of parafollicular cells with variable shaped nuclei were also encountered.

However, morphometric studies of both males and females of the tamoxifen group revealed non significant change in their

follicular surface area and perimeter when compared to the control group.

# Annex

## 1-Light microscopic study

***(Drury and Wallington, 1980)***

### Processing of sections for light microscopic examination:

*The specimens were processed as follows:*

1. The specimens were fixed in 10% buffered neutral formalin for at least 7 days.

2. They were, then, transferred to ascending grades of ethyl alcohol (50%, 70%, 96% and absolute alcohol) for dehydration.

3. The specimens were cleared in xylol.

4. The tissues were, then, impregnated with soft paraffin by putting them in several changes of melted wax (melting point 50°C) in an oven.

5. Finally, embedding in hard paraffin was done by putting the tissues in melted wax (melting point 55°C), poured into a mold and then cooled down to form paraffin blocks containing the tissues.

6. Serial sections 5-7 μm thick were sliced, mounted on slides with an adhesive, stained and examined with an Olympus light

microscope equipped with an automatic digital photomicrographic camera system.

## 1.Hematoxyline and Eosin stain:

The sections were:

1. Taken down to water.
2. Stained with hematoxyline for two minutes.
3. Washed well in tap water for ten minutes.
4. Rinsed in distilled water.
5. Stained with eosin for one minute.
6. Put in distilled water for seconds.
7. Dehydrated in ascending grades of ethyl alcohol.
8. Cleared in xylol.
9. Mounted in Canada balsam.

*Results:*

- The nuclei were stained blue.
- The cytoplasm was stained deep pink.

## 2.Masson's trichrome stain:

**(modified from Masson 1929)**

The sections were:

1. Taken to water.
2. Stained nuclei with iron haematoxylin.
3. Washed well in water.
4. Differentiated nuclear stain with 0.5 per cent hydrochloric acid in 70 per cent alcohol.
5. Washed well in tap water, rinse in distilled water.
6. Stained in the red cytoplasmic stain 5-10 minutes.
7. Rinsed in distilled water.
8. Differentiated in 1 per cent phosphomolybdic acid until collagen is decolorized, muscle, red blood cells and fibrin remaining red.
9. Rinsed in distilled water.
10. Counterstained in antiline blue for 2-5 minutes.
11. Washed well in 1 per cent acetic acid for at least one minute.
12. Blotted, dehydrated in absolute alcohol, cleared in xylene, mounted in a synthetic resin medium.

*Results:*

-Nuclei were stained black.

-Muscle, red blood cells, fibrin and some cytoplasmic organelles were stained red.

-Collagen was stained blue or green

## 2-Electron microscopic study

### Processing of sections for transmission electron microscopic examination:

1. Small pieces of the thyroid gland (1mm$^3$) were fixed in 3% phosphate buffered Glutraldehyde (pH 7.3) for overnight at 4°C ***(Sabatini et al., 1963)***.

2. After two rinses in the phosphate buffer (for a period of 4 hours), the specimens were post fixed in 1% buffered osmium tetroxide for 1-2 hours at 4°C ***(Millonig, 1961)***.

3. The tissue pieces were then washed twice in phosphate buffer for 30 minutes each.

4. After that the specimens were dehydrated in ascending grades of ethyl alcohol (50%, 70%, 80%, 90% and absolute alcohol) two times in each grade for 15 minutes but 30 minutes in the absolute alcohol.

5. Clearing was done in propylene oxide for 20 minutes at room temperature.

6. Infiltration was then done by using equal parts of propylene oxide and Epon 812 for overnight.

7. Finally, the specimens were embedded in gelatin capsules filled with fresh Epon. The capsules were placed in the oven at 60°C for 48 hours to allow polymerization ***(Luft, 1961)***.

8. Semithin sections were cut at 1 μm thickness with a glass knife. They were stained with 1% toluidine blue stain dissolved in 1% borax for approximately 30-60 seconds at 60-70°C and examined by the Olympus light microscope for general orientation ***(Bancroft and Gamble, 2002)***.

9. Ultrathin sections (50-60 nm thick) were cut from the selected regions using an LKB ultramicrotome with a diamond knife. The sections were picked on uncoated 200 mesh copper grids.

10. After that the grids were stained and examined by *Sumy Electron Optics - Video Imaging System, PEM-100* electron microscope at 60-80 kv in Transmission Electron Microscope Unit at Medical Military Academy. Finally, electron micrographs were taken from the selected areas.

## Double stain method for ultrathin sections

The grids were:

1. Stained with a drop of saturated solution of uranyl acetate for 7 minutes ***(Swift and Rash, 1958)***.
2. Rinsed in distilled water.
3. Then stained with a drop of lead citrate for another 7 minutes ***(Reynolds, 1963).***
4. Rinsed in distilled water.
5. Put on a filter paper and left to dry.

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