Histological and Biochemical Study on the Toxic Effects of Bisphenol A on the Thyroid Gland of Adult Male Albino Rats and the Possible Protection by Selenium

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ABSTRACT

Background: The worldwide used bisphenol A (BPA) which is incorporated in many plastic industries is considered as an endocrine disruptor. Warnings raised by many agencies against the excessive use of such substances because of its confirmed hazardous effects. Selenium (Se) is known to have antioxidant role in living systems.

Aim of the work: The aim of the current study was to evaluate the extent to which BPA can affect the thyroid gland structure and function and if there is any protective role for selenium.

Material and Methods: Twenty four adult male rats divided equally into three groups. Group I as a control, group II included rats that received 50 mg/kg day bisphenol orally for eight weeks and group III that received bisphenol in the same dose for the same duration concomitantly with selenium in a dose of 0.5 mg/kg. At the end of the experiment, blood samples were taken for hormonal assay and statistical analysis. Thyroid gland tissue samples were processed for histological and immunohistochemical study.

Results: BPA-treated rats showed degenerative changes in the form of decreased or absent colloid and fusion of the follicles. Other follicles showed signs of hyperactivity as microfollicles, follicular epithelial cell stratification and many lysosomes. Strong positive inducible nitric oxide synthase immunoreactivity and highly significant increase of serum TSH with decreased T3 and T4 were recorded. BPA+selenium treated group revealed nearly normal appearance of thyroid gland architecture.

Conclusion: BPA had deleterious effects on thyroid structure and function. Concomitant administration of selenium preserved to a great extent the thyroid gland architecture.

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Key Words: Bisphenol, rat, selenium, thyroid gland.

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INTRODUCTION

Bisphenol A (BPA) is the molecular building block for epoxy resins and polycarbonate plastics. It became one of the most produced chemicals in the world due to rapid growth of its production. It is now found nearly everywhere in the environment and commonly detected in surface water, drinking water and dust particles. Moreover, exposure of the consumer to BPA can occur via the diet. Human exposure can result from multiple sources, particularly from the direct contact of food with plastics containing BPA. The lining of food and drink cans which was derived from BPA leaching from the plastic material has received particular attention. The attention was focused on other routes of exposure include BPA leaching from baby feeding bottles and BPA related compounds leaching from microwave plastic containers, polycarbonate plastic bottles and reusable water bottles. Moreover, compounds leaching from plastic wraps, food storage containers, paper towels, DVDs, computers, dental fillings, sealants, home appliances, spectacles, optical lenses and sports safety equipment were considered BPA related compounds. BPA has been found in humans breast milk, urine, blood and tissues. It was found to rise multiple diseases including cancer breast, decline in semen quality in men, urogenital abnormalities in male babies, early onset of puberty in girls, neurobehavioral problems, obesity and metabolic disorders including type 2 diabetes. Furthermore, bisphenol-A can change synthesis of endogenous hormone, hormone concentrations in blood and hormone metabolism. BPA has agonistic effect towards the estrogenic receptor and estrogenic properties as confirmed by numerous biochemical and toxicological studies hence, it mimics the estrogen role once it enters into living systems. Therefore, BPA belongs to a group of chemicals termed “Endocrine disruptors” or “Hormone disruptors”.

Adverse effects of BPA have been shown by several studies on laboratory animals on reproductive system, brain and metabolic processes. Moreover, BPA absorption in large amounts through skin has been shown to cause marked damage to kidney, liver and other vital organs in human. Oxidative toxicity has been reported by several studies after exposure to BPA in rats and mice.
Tissue injury in the brain, kidney, liver and other organs is caused by BPA via the formation of reactive oxygen species (ROS) through increasing lipid peroxidation and decreasing antioxidant enzymes activities, thereby causing oxidative stress\cite{18,19}.

Selenium (Se) is a sulphur analogue which is an essential trace element and its low status has been associated with increased risk of various diseases in humans. Research on Se has attracted great interest because of its important antioxidant role for protection against oxidative stress which is initiated by excess reactive oxygen species (ROS) and reactive nitrogen species (NOS). During the last few years, Se research has proved the important role that Se and its metabolites play in human diseases. In particular, the knowledge of the functional roles of enzymes: glutathione peroxidase and other peroxidases and some selenoproteins\cite{20}. Selenium is active immunomodulator which is much more potent anti-oxidant than vitamins E, A and C, beta-carotene, but much more toxic. It participates in conversion of thyroxine to triiodothyronine in biosynthesis of thyroid hormone. Selenium is considered as a serious factor of biological and antioxidant protection of vascular endothelium, DNA, chromosomes and low-density lipoproteins. Selenium as food component is an exceptional agent of protection from atherosclerosis, coronary ischemic disease and cancer. Kidney, liver, corn and cabbage, broccoli, garlic, onion, are dietary products with high selenium content\cite{21,22}.

As to date, a controversy about the toxicity of BPA is present. Although BPA has been labeled as a safe agent by FDA\cite{23}, newly emerging data has reported more studies on human health risk assessment of BPA exposure\cite{24} especially where plastic usage has exponentially increased as in developing countries. Certain population groups such as those suffering from malnutrition may be at higher risk than other populations\cite{25,26}.

Accordingly, the aim of this study was to detect the effects of BPA administration on thyroid gland and to evaluate selenium role as a well known antioxidant in protection of the gland against these hazardous effects.

**MATERIAL AND METHODS**

**Animals**

This study was conducted on 24 adult male albino rats (3 months) weighing 180–200 g. The rats were obtained from the animal house of the Faculty of Medicine, Assuit University; Housing of the rats was in stainless-steel cages and they were maintained in room temperature at 23°C. They were fed a standard diet and allowed at water ad libitum and, this experiment was accomplished with the standard guidelines of animal ethics committee (Assiut University) in accordance with principles accepted internationally for the laboratory animal care and use.

**Chemicals and drugs**

BPA (purity >99%) was purchased from Sigma-Aldrich Co. (St Louis, Missouri, USA). It was dissolved in corn oil (vehicle) and given to rats at a dose of 50 mg/kg body weight orally once daily for eight weeks. The dose of BPA was equal to the lowest dose which is commonly used to refer to the environmentally relevant doses. Such doses resulting in serum levels close to those observed in human serum\cite{27}. Selenium (Na2SeO) was also purchased from the same company.

Inducible nitric oxide synthase (iNOS) was purchased from Thermo Scientific Company, USA.

**Experimental design**

The rats were equally divided into three groups (8 rats each): the control group (group I), and two experimental groups (groups II and III).

Group I (control): Rats in this group received oral administration of corn oil (vehicle) daily throughout the experimental period.

Experimental group II (BPA-treated group): These rats received BPA orally in a dose of 50 mg/kg body weight respectively dissolved in corn oil for 30 days\cite{27}.

Experimental group III (BPA+selenium treated group): The rats received the same dose of BPA for the same duration as group II with concomitant administration of selenium in a dose of 0.5 mg/kg\cite{28}.

**Blood sample collection**

At the end of the experiment, blood samples from the rats’ tails of group I, II and III were collected into clear sterile tubes, then the blood was centrifuged at 3000 round per minute (rpm) for 20 min. Sera were collected and stored at -20°C for hormonal assay.

**Tissue sample collection**

At the end of the experimental period, the rats were anaesthetized with ether inhalation. Incision in the mid line was done to identify sternomastoid and sternohyoid muscles. The trachea was exposed by separation of these muscles. The trachea was traced upward gently until the thyroid glands were visible. It appeared as two small oval reddish masses on each side of the trachea. The glands were dissected gently to avoid their injury\cite{29}. The thyroid glands were obtained and divided into two parts. One part was processed for light microscopic study using Haematoxylin and Eosin (H&E) and Masson’s trichrome stains. For immunohistochemical study by using inducible nitric oxide synthase (iNOS), some sections were used. The second part was processed for electron microscopic study.

**Histological study**

For examination by light microscope, specimens were fixed in neutral buffer formalin saline 10% for 24 hours and were processed for preparation of paraffin sections 5 μm thickness to be stained with H&E and Masson’s trichrome stains\cite{30}.
For examination by electron microscope, specimens were cut into thin slices (1×1 mm) and immediately immersed in the same perfusion fixative (4% glutaraldehyde solution for 20 h), then the specimens were washed with buffer solution and fixed in osmium tetroxide 1% concentration buffered with 0.1 M phosphate buffer at 7.4 pH for 1 hr. Then, semithin sections (0.5-1 μm) were prepared and stained with 1% toluidine blue. By using an ultramicrotome (Leica, Glieneck, Berlin, Germany), ultrathin sections (80-90 nm) were obtained. Sections were stained with uranyl acetate and lead citrate\(^{(1)}\). Ultrathin sections examined and photographed with transmission electron microscope (Jeol- JEM- 100 CXII; Jeol, Tokyo, Japan) in the Electron Microscopic unit, Assuit University (Egypt).

**Immunohistochemical study**

Immunohistochemical staining for detection of inducible nitric oxide synthase (iNOS) was demonstrated using Labeled Streptavidin–Biotin immunoperoxidase technique with antibody against the iNOS marker for oxidative stress. The sections were deparaffinized in xylene, rehydrated, washed in 0.1 mol/l PBS, treated with 0.01% trypsin at 37°C for 10 min, and then washed for 5 min with PBS. Treatment with 0.5% hydrogen peroxidase (H\(_2\)O\(_2\)) for 5 minutes was done for endogenous peroxidases blocking. Immunoperoxidase stain for iNOS (Polyclonal Rabbit Anti-iNOS) was used to recognize iNOS which is detected in the cytoplasm. Sections were incubated with the primary antibody (1:100) overnight for 60 minutes at 4°C. The samples washed twice with PBS before applying the secondary antibody. The universal kit used was biotinylated secondary antibodies. Expressions were visualized using diaminobenzidine tetrahydrochloride for 5-minutes (Sigma, St. Louis, MO) treatment. Counterstaining of the slides was done with Mayer’s hematoxylin before mounting with D.P.X\(^{(2)}\). For negative controls, the primary antibody recognizing iNOS was omitted by using normal rabbit serum (×100 diluted) instead of the primary antibody. The positive control was a lung slide.

**Measurement of serum hormone levels**

Serum hormone levels of T3, T4 and TSH were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Measurement of serum TSH, total T3 and total T4 concentration are generally regarded as a valuable tool in the diagnosis of thyroid dysfunction; Kits were used (Monobid Inc. lake forest CA 92630,USA).

**Statistical study**

Expression of data for all groups was as mean ± standard deviation (X±SD). The obtained data was subjected to SPSS program, version 15. Determination of statistical significant difference was by one way analysis of variance (ANOVA). The values of probability \(P<0.05 <0.001\) and \(>0.05\) were considered significant, highly significant and non-significant, respectively.

**RESULTS**

**Histological and immunohistochemical results**

**Control group**

**Light microscopic examination**

HandE-stained sections from the thyroid glands showed the thyroid parenchyma was composed of different sized follicles, where large follicles were present, especially at the periphery. The follicular wall was lined by a single layer of flattened to cuboidal follicular cells, with oval to round nuclei. The follicular lumen was filled with homogenous acidophilic colloids that had peripheral small vacuoles. An apparent few number of interfollicular cells and blood capillaries were observed in between the follicles (Figures 1 and 2).

Toluidine blue-stained semithin sections showed that the follicles of thyroid gland were lined with a single layer of follicular epithelium. The follicular cells revealed rounded to oval nuclei. Pale large parafollicular (C) cells with small granules were observed. Fibroblasts with dark elongated nuclei and also small blood capillaries appeared in between the follicles (Figure 3).

Masson’s trichrome stain revealed very scanty green colored collagen fibers between the thyroid follicles (Figure 4).

Immunohistochemical study showed weak immune reaction for inducible nitric oxide synthase (iNOS) in the follicular cells (Figure 5).

**Electron microscopic examination**

Ultrastructurally, some follicular cells were active with cuboidal epithelium. They had oval nuclei with clumps of heterochromatin. Their cytoplasm had mitochondria, rough endoplasmic reticulum cisternae and moderate to large electron dense cytoplasmic colloid droplets. Their apical border showed a moderate or a small number of microvilli projecting into the colloid. Lateral surfaces of follicular cells show tight junctions (Figure 6). Other active follicular cells had high cuboidal or columnar epithelium with euchromatic rounded nuclei resting on basal thin lamina with with blood vessels indenting it. The cytoplasm revealed numerous rough endoplasmic reticulum cisternae which are regular and parallel, mitochondria and dense lysosomal granules. The apical borders had microvilli in a huge number projecting into the colloid (Figure 7). Less common cells were parafollicular or C cells with oval euchromatic nucleus. These cells were separated from the luminal colloid by a part of more or less inactive flat follicular cell cytoplasm with noticeable rough endoplasmic reticulum streaks, mitochondria, moderate electron dense cytoplasmic colloid droplets and lysosomes. Note that the apical border had a moderate or a small number of microvilli projecting into the colloid (Figure 8).
**Bisphenol -treated group**

**Light microscopic examination**

Bisphenol-treated group stained with H andE showed drastic loss of normal thyroid architecture. The acini showed irregular shape and size with microcystic follicles with absent and scanty amount of colloid. Some of the follicles appeared degenerated, others appeared with exfoliated desquamated cells in the lumen and some appeared fused (Figure 9). Other follicles appeared with no colloid or with peripheral scalloping and vacuolated follicular cells. Some follicles revealed disrupted basal lamina with coalescence of the follicles and others appeared with multiple layers that obliterated the lumen (Figure 10).

Toluidine blue stained sections revealed that some of the follicles appeared with completely obliterated lumen and multiple cell layers follicular cells obliterating the lumen. These cells revealed mostly irregular nuclei or highly condensed nuclei (Figure 11). The follicles showed an apparent increase in the height of their lining epithelium. The lining follicular cells were lined with darkly stained irregular corrugated nuclei with an extensive vacuolated or foamy cytoplasm. Numerous blood capillaries can be seen, some of them indenting the follicular epithelium with large congested blood vessels can be observed. Also, large parafollicular cells with a rounded pale nucleus and a pale cytoplasm can be seen within the follicular epithelium and in the interfollicular connective tissue (Figure 12).

Sections stained with Masson’s trichrome stain showed an increased greenish colored collagen fibers deposition between severely disrupted thyroid follicles compared with those of the control group (Figure 13).

Moreover, iNOS immunostained stained sections revealed a very strong positive immunoreactivity for iNOS as a brown cytoplasmic immune reaction in the follicular cells (Figure 14).

**Electron microscopic examination**

Bisphenol-treated group examination showed flat follicular cells resting on the basal lamina with a heterochromatic irregular nucleus and severe indentation of the outer nuclear membrane. Their cytoplasm showed numerous markedly dilated rough endoplasmic reticulum, multiple dense lysosomal granules, small scattered vacuoles and small electron dense mitochondria. The apical borders showed long microvilli in a huge number. Widening of the lateral junction between thyrocytes is noticed (Figure 15). Some columnar or high cuboidal follicular cells revealed irregular nuclei with peripheral heterochromatin clumping. Other follicular cells had apoptotic nuclei and their cytoplasm showed numerous vacuoles, dilated irregular rough endoplasmic reticulum cisternae, dense cytoplasmic droplets, lysosomal granules with large number of aggregated microvilli among the apical borders (Figures, 16, 17 and 18). On the other hand, some thyroid follicular cells cytoplasm declared excess inclusion deformed lysosomes, markedly dilated rough endoplasmic reticulum and electron dense elongated mitochondria with loss of its pattern. Extensive microvilli on the apical border and widening of the lateral junction were observed (Figure 19). Ultrastructural examination of the parafollicular cells showed rounded euchromatic nuclei with peripheral heterochromatin. Numerous, small high-density secretory granules and numerous small mitochondria occupy a large portion of their cytoplasm. They are separated from the luminal colloid by a part of the cytoplasm of follicular cell. The follicular cell apical portion can be seen with an irregular apoptotic nucleus, dilated rough endoplasmic reticulum cisterna and small numerous, dense lysosomal granules (Figures 15 and 20).

**Bisphenol +selenium treated group**

**Light microscopic examination**

Light microscopio examination of the thyroid gland of the bisphenol +selenium treated rats showed nearly normal histological appearance compared to the pvious group where with using HandE staining showed that most of the thyroid follicles almost restored their normal architecture except some of them still showed complete obliteration of the lumen, some follicles appeared with peripheral scalloping of their colloid (Figures 21 and 22).

With toluidine blue staining, the thyroid follicles appeared more or less similar to the control. However still some follicles show high cuboidal to columnar follicular cells. Others revealed irregular dense nucleus and foamy cytoplasm. The blood vessel appeared indenting the basal lamina (Figure 23).

Sections stained with Masson's trichrome stain showed little collagen deposition between thyroid follicles in comparison with thyroid in group II and appeared nearly similar to the control group (Figure 24).

Immunostained sections revealed a slight cytoplasmic immune reaction for iNOS (Figure 25).

**Electron microscope examination**

Ultrathin sections of thyroid gland in the protected group showed flat thyrocytes which appeared more or less similar to the control. It revealed flat euchromatic idented nucleus with peripheral rim of heterochromatin, moderately dilated rough endoplasmic reticulum cisternae normal appearance of lysosomes, mitochondria and colloid in the lumen. Still there was disruption of microvilli and loss of most of them. Note nearly normal appearance of the lateral junction among thyrocytes (Figures 26 and 27), however still there is slight widening of the lateral junction between some thyrocytes. Moreover, examination of the parafollicular cells revealed euchromatic nucleus, mitochondria and small electron-dense secretory granules (Figure 28).

**Hormonal essay Morphometric results and statistical analysis**

Statistical analysis of T3 and T4 serum level in
bisphenol-treated group revealed T3 and T4 decrease in this group which was found to be highly significant \((P<0.001)\) in comparison with the control group. However, the TSH hormone was increased in bisphenol treated group in comparison to control one and the difference was found to be statistically highly significant. On the other hand, the recorded data in selenium-treated group were found approaching the control level and the detected difference was found to be statistically non-significant (Table 1, Histogram I, II and III).

![Figure 1](image1.png) **Fig. 1:** A photomicrograph of a section in the thyroid gland of a control rat (group I) showing follicles of various sizes (F). Its walls are lined by follicular cells surrounding colloid (C) inside its lumina. Interfollicular cells (arrow heads) and blood capillaries (arrows) are shown between the follicles. Part of the parathyroid gland (Pa) can be also noticed. H&E, X200.

![Figure 2](image2.png) **Fig. 2:** A photomicrograph of a section in the thyroid gland of a control rat showing the follicular walls lined by flattened (thin arrow) to cuboidal (thick arrows) follicular cells with oval to rounded nuclei. The follicular lumen is filled with homogenous acidophilic colloids (C) that show peripheral small vacuoles (V). An apparent few number of interfollicular cells (arrow head) and blood capillaries (curved arrows) can be observed in between follicles. H&E, X400.

![Figure 3](image3.png) **Fig. 3:** A photomicrograph of a semithin section in the thyroid gland of a control rat showing that the thyroid follicles are lined by a single layer of follicular epithelium. The lining follicular cells appear with rounded (N) to oval nuclei (n). Fibroblasts with dark elongated nuclei (arrow head) and small blood capillaries (arrows) can be seen between the follicles. Also, a large pale parafollicular cell that indented the basement membrane with small dense granules (P) can be observed. Toluidine blue, X1000.

![Figure 4](image4.png) **Fig. 4:** A Photomicrograph of section in the thyroid gland of a control rat showing minimal green colored collagen fibers between the follicles of thyroid (arrows). Masson's trichrome, X 400.

![Figure 5](image5.png) **Fig. 5:** A Photomicrograph of a section in the thyroid gland of a control rat showing weak immune reaction (short arrows) for inducible nitric oxide synthase (iNOS). iNOS, x400.
Fig. 6: An electron micrograph of ultrathin section in the thyroid gland of a control rat showing cuboidal follicular cells with oval irregular nuclei (N) containing clumps of heterochromatin. These cells rest on basal lamina (arrow). Their cytoplasm shows mitochondria (m), cisternae of rough endoplasmic reticulum (rER) and moderate to large electron dense cytoplasmic colloid droplets (d). Their apical borders shows moderate or small number of microvilli (mv) projecting into the colloid (C). Lateral surfaces of follicular cells show tight junctions (arrow heads). TEM, X7200.

Fig. 7: An electron micrograph of ultrathin section in the thyroid gland of a control rat showing follicular cells with high cuboidal epithelium that reveals euchromatic more or less rounded nucleus (N) with peripheral chromatin condensation resting on thin basal lamina (arrows) which is indented with blood vessel (bv). The cytoplasm has numerous paralleled cisternae of rough endoplasmic reticulum (rER), dense lysosomal granules (L) and mitochondria (m). Their apical borders show a huge number of microvilli (mv) projecting into the colloid (C). TEM, X5800.

Fig. 8: An electron micrograph of ultrathin section in the thyroid gland of a control rat showing parafollicular or C-cells with oval euchromatic nucleus (N) lying on the basal lamina (arrow). Their cytoplasm contains mitochondria (M), small electron-dense secretory granules (g) and rough endoplasmic reticulum few tubular cisternae (arrow head). The cell is separated from the luminal colloid by a part of the cytoplasm of a flat follicular cell containing heterochromatin nucleus (n*) with prominent nucleolus (nu), rough endoplasmic reticulum (rER), mitochondria (m), moderate electron dense cytoplasmic colloid droplets (d) and lysosomes (L). Note the apical border showing moderate or small number of microvilli projecting into the colloid (mv). TEM, 7200.

Fig. 9: A Photomicrograph of a section in the thyroid gland of bisphenol-treated group (group II) showing follicular loss of normal architecture (**). The acini showed irregular shape and size with microcystic follicles (F) with absent or scanty amount of colloid (C). Some of the follicles appeared degenerated (D), others appeared with cells which are desquamated in the lumen (arrow head) and some appeared fused (arrow). HandE, X 200.
Fig. 10: A photomicrograph of a section in the thyroid gland of group II showing marked disruption of the normal appearance where some follicles with no colloid (F) others with peripheral scalloping (PS) and vacuolated follicular cells with loss of their nuclei. Other follicles appeared with obliterated lumen (F1) or disrupted basal lamina (arrow heads). Others appeared with multiple layers (*) and vacuolated cytoplasm (curved arrows). Congested blood capillaries are observed (arrow). HandE, X400.

Fig. 11: A photomicrograph of a semithin section in the thyroid gland of group II showing multiple layers of follicular cells lining the follicle obliterating its lumen (thick arrow). These cells are with mostly irregular nuclei (N) or highly condensed nuclei (arrow heads) and severely vacuolated cytoplasm (V) can be seen. Toluidine blue, X1000.

Fig. 12: A photomicrograph of a semithin section in the thyroid gland of group II showing a follicle (F) with an apparent increase in the height of their lining epithelium. The lining follicular cells are lined with darkly stained irregular corrugated nuclei (n) with an extensive vacuolated cytoplasm (V) or foamy cytoplasm (arrow head). Numerous blood capillaries can be seen, some of them indenting the follicular epithelium (bv) and others are large congested blood vessels (bv*). Also, large parafollicular cells (p) with a rounded pale nucleus and a pale cytoplasm can be seen. Some of the follicles appear with completely obliterated lumen with multiple cell layers (*). Toluidine blue, X1000.

Fig. 13: A photomicrograph of a section of group II showing increased greenish colored collagen fibers deposition between severely disrupted thyroid follicles (arrows) compared with those of the control group. Masson's trichrome, X400.
ROLE OF SELENIUM ON THYROID TOXICITY INDUCED BY BISPHENOL A.

Fig. 14: A Photomicrograph of a section in the thyroid gland of group II showing a very strong positive immunoreactivity for iNOS as a brown cytoplasmic immune reaction in the follicular cells (short arrows). iNOS, X400.

Fig. 15: An electron micrograph of ultrathin section in the thyroid gland of group II showing flat follicular cells having a heterochromatic irregular nucleus (N) with sever indentation of the outer nuclear membrane (curved arrow) resting and these cells rest on the basal lamina (arrow head). The follicular cells show numerous markedly dilated rough endoplasmic reticulum (rER). Their cytoplasm shows small electron dense mitochondria (m), multiple dense lysosomal granules (L), and scattered small vacuoles (V). Their apical borders show a huge number of long microvilli (mv). Note widening of the lateral junction (arrows) between the thyrocytes. A part of a para follicular cell is noticed showing a part of an euchromatic nucleus (n). Its cytoplasm contains numerous small electron-dense secretory granules (g) and numerous small mitochondria (m). A part of the follicular cell cytoplasm separates this cell from the luminal colloid. TEM, X7200.

Fig. 16: An electron micrograph of ultrathin section in the thyroid gland of group II showing high cuboidal or columnar follicular cells. These cells have apoptotic nuclei (n) or irregular nuclei (N) with peripheral heterochromatin clumping. Small electron dense mitochondria (m) numerous dilated irregular rough endoplasmic reticulum cisternae (rER), numerous vacuoles (V) and dense lysosomal granules (L) can be seen. Their apical borders show aggregated microvilli in a huge number (mv). TEM, X5800.

Fig. 17: An electron micrograph of ultrathin section in the thyroid gland of group II showing follicular cells which are arranged in layers. Some of these cells, have euchromatic nuclei (N) with irregular outline and peripheral condensation of hetrochromatin, others appeared with apoptotic nuclei (n). Numerous dilated rough endoplasmic reticulum (rER), Lysosomal granules (L) and multiple vacuoles (V) can also be seen. TEM, X5800.
Fig. 18: An electron micrograph of ultrathin section in the thyroid gland of group II showing follicular cells. Some cells have irregular large nucleus (N) with peripheral chromatin condensation and irregular nucleolus (nu). The cytoplasm revealed distended irregular rough endoplasmic reticulum (rER), numerous lysosomes (L) and dense cytoplasmic droplets (d). Notice the microvilli (mv) on the apical border and colloid (C). TEM, X5800.

Fig. 19: An electron micrograph of ultrathin section in the thyroid gland of group II showing follicular cells containing irregular nuclei with peripheral condensation of heterochromatin (N), inclusion deformed lysosomes (L), electron dense elongated mitochondria with loss of its pattern (m) and markedly dilated rough endoplasmic reticulum (rER). Note the extensive microvilli (mv) on the apical border, colloid (C) in the lumen and widening of the lateral junction (arrow heads). TEM, X5800.

Fig. 20: An electron micrograph of ultrathin section in the thyroid gland of group II showing a parafollicular cell with multiple nuclei (N) revealing peripheral heterochromatin. Numerous small high-density secretory granules (g) and numerous small mitochondria (M) occupying a large portion of the cytoplasm are seen. The cell is separated from the luminal colloid (C) by a part of follicular cell cytoplasm (*) which reveals an irregular apoptotic nucleus (n), numerous small dense lysosomal granules (L) and dilated rough endoplasmic reticulum cisternae (rER). TEM, X5800.

Fig. 21: A photomicrograph of a section in the thyroid gland of bisphenol + selenium treated rats (group III) showing that most of the follicles of thyroid (*) almost restore their normal architecture except some of them still show complete obliteration of the lumen (arrow). H&E, X200.
ROLE OF SELENIUM ON THYROID TOXICITY INDUCED BY BISPHENOL A.

Fig. 22: A Photomicrograph of a section in the thyroid gland of group III showing normal architecture restoration of the thyroid gland follicles (F). Few follicles appear with peripheral scalloping (PS) in their colloid. HandE, X400.

Fig. 23: A Photomicrograph of a semithin section in the thyroid gland of group III showing thyroid follicles (F) which appear more or less similar to the control. However still some follicles show high cuboidal to columnar follicular cells (arrow). Others reveal irregular dense nucleus (N) and foamy cytoplasm (arrow head). Note the blood vessel (bv) indenting the basal lamina. Toluidine blue, X1000.

Fig. 24: A photomicrograph of section in the thyroid gland of group III showing little deposition of collagen (arrows) which appears more or less normal as compared with that in group II. Masson's trichrome, X400.

Fig. 25: A photomicrograph of section in the thyroid gland of group III showing a slight cytoplasmic immune reaction for iNOS (short arrows). iNOS, X400.

Fig. 26: An electron micrograph of ultrathin section in the thyroid gland of group III showing flat follicular cell which appears more or less similar to the control. It revealed flat euchromatic indented nucleus (N) with peripheral rim of heterochromatin. Note and colloid in the lumen (C) and small number of microvilli (arrow head). The cytoplasm contains mitochondria (m), lysosomes (L) and rough endoplasmic reticulum sticks (rER). TEM, X7200.

Fig. 27: An electron micrograph of ultrathin section in the thyroid gland of group III showing high cuboidal follicular cells with indented heterochromatin nucleus (N). Their cytoplasm contains moderately dilated rough endoplasmic reticulum cisternae (rER), lysosomes (L) and nearly normal appearance of microvilli (mv). Note more or less normal appearance of the lateral junction (arrow). TEM, X5800.
Fig. 28: An electron micrograph from the thyroid gland of group III showing a parafollicular cell containing euchromatic nucleus (N), small electron-dense secretory granules (g) and mitochondria (m). This cell is separated from the luminal colloid by a part of follicular cell cytoplasm (*). Note the follicular cells with euchromatic nucleus (n), few dilated rough endoplasmic reticulum cisternae (rER) and lysosomes (L), however still there is slight widening of the lateral junction between the thyrocytes (arrow head). TEM, X5800.

Table 1: Comparison between TSH, T3 and T4 levels (ng/ml) in the different studied group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thyroid Hormones</th>
<th>Control group</th>
<th>BPA group</th>
<th>BPA+ Selenium group</th>
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<tr>
<td>TSH</td>
<td>1.76 ± .052</td>
<td>3.81 ± 1.80a</td>
<td>1.91 ± 1.56b</td>
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<tr>
<td>T4</td>
<td>4.95 ± .151</td>
<td>1.10 ± .054a</td>
<td>2.84 ± .199b</td>
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<tr>
<td>T3</td>
<td>42.00 ± 6.19</td>
<td>18.75 ± .793a</td>
<td>38.30 ± .731b</td>
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</tr>
</tbody>
</table>

a Significant as compared with the control group p≤0.001
b Significant as compared with BPA+ Selenium group p≤0.001

Histogram 1: A graphical representation for comparison between TSH level ng/100ml in different groups

Histogram 2: A graphical representation for comparison between T4 level ng/100ml in different groups

Histogram 3: A graphical representation for comparison between T3 level ng/100ml in different groups

DISCUSSION

Thyroid hormones have important roles in normal growth, behavioural, intellectual neuronal development and sustaining metabolic homeostasis. Diminished thyroid hormone function may affect these crucial functions[33]. Because of its structural similarity to thyroid hormone BPA may have an action as an agonist or antagonist of the thyroid hormone receptor. Hence, thyroid hormone receptors are expressed plentifully in different organs, BPA may disrupt thyroid hormone action throughout the body tissue. BPA was revealed to suppress T3 and disturb thyroid homeostasis[34]. It is a well-known an endocrine-disrupting
ROLE OF SELENIUM ON THYROID TOXICITY INDUCED BY BISPHENOL A.

The present study revealed that the current study declared that the thyroid follicular cells[35]. The recent years declared an increase in the number of industrial applications of BPA products. Automotive manufacturers have started substituting glasses with polycarbonate plastics made from BPA[36]. Because of its common use, and resulting pollution of the environment, human exposure to BPA is unavoidable and universal. Much attention has been raised up among scientists and governing agencies about probable adverse health consequences related to these continuous, life-long exposures to BPA[37].

The present study declared that hormonal essay of the BPA-treated group showed significant reduction of T3 or T4 in comparison to the control group which exerts a negative feedback on the pituitary gland and causes release of more TSH which also revealed highly significant increase in comparison to the control group. This increase is mostly to stimulate the thyroid gland, in a trial to accelerate the production of the thyroid hormone. These findings are in agreement with other researchers[38, 39 and 40] who found a significant decrease in plasma triiodothyronin (T3), thyroxin (T4) that was recorded after 14 days BPA exposure. Moreover, the current results came in line with the results of Wang et al.[41] who declared that the exposure of BPA also alter thyroid functions, leading to thyroid abnormalities, such as subclinical hypothyroidism. The present findings comes in harmony with, the results of Jassim[42] who found a significant decrease in T4 in BPA-treated group in comparison to the control one. TSH significant increase in the level of TSH hormone in BPA-treated group in comparison to the control one. TSH stimulates the growth of the gland; thus, the gland becomes enlarged as TSH is known to be the main growth factor for thyroid[44]. The gland under TSH undergoes enlargement, neovascularization, hyperplasia, and morphological changes in the thyrocytes[45].

In this study, some follicular lumina of BPA-treated group showed desquamated follicular cells. In accordance, some researchers[46] reported that the degenerated follicular epithelial cells are susceptible to slough off. Furthermore not only degenerative follicular cells were detected but also detached apical parts of the cytoplasm of other follicular cells. These changes could be attributed to cellular distension with accumulated colloid which resulted in cellular disruption[51, 52 and 53]. The present study revealed significant increase in the level of TSH hormone in BPA-treated group in comparison to the control one. TSH stimulates the growth of the gland; thus, the gland becomes enlarged as TSH is known to be the main growth factor for thyroid[44]. The gland under TSH undergoes enlargement, neovascularization, hyperplasia, and morphological changes in the thyrocytes[45].

Features of hyperactivity in the present study were found in some thyroid follicles as extensive vacuolations of colloid and colloidal droplets in follicular cells. These vacuolations were attributed to increased endocytotic activities that release the stored hormones as a compensatory mechanism to BPA mediated suppressive effects on follicular cells. It was also hypothesized that these vacuolations were under the influence of increased TSH[44]. This elucidation was in accordance with other researchers[56] who stated that during the huge demand for thyroid hormones, follicular cells spread out pseudopods into the lumen of the follicles to enclose and absorb the colloid. Furthermore, BPA-treated thyroid in this work showed an increase in the height of follicular epithelium. This was in agreement with the results of many researchers[57] who found a significant increase in the height of the epithelium in hypothyroidism.

In this study, hyperplasia, epithelial stratification, microfollicles, increase vascularity and hypertrophy of follicular cells were observed. These could be attributed to an increase in the level of TSH, which was responsible...
for the follicular cells proliferative activity\cite{57, 58}. BPA itself is mutagenic; it can cause uncontrolled proliferation of cells\cite{59}. Hypertrophy is an increase in cell size and functional capacity; when trophic signals or the functional demand increase, adaptive changes occur to fulfill these needs\cite{60}. This hypertrophic change is because of the hydropic swelling resulting from impairment in cellular volume regulation. The detrimental agent may interfere with membrane-regulated processes by increasing plasma membrane permeability to sodium or destruction of the pump directly or interfering with the synthesis of ATP\cite{60, 61}.

Proliferation of microvillous borders and increased number of lysosomes with their apical condensation were observed in this work. These ultrastructural findings of hyperactivity were in agreement with those reported by other researchers\cite{61}. This proliferation of microvillus border facilitates the transport and iodination of thyroglobulin across the cell membrane\cite{62}.

In the current study, the BPA-treated group showed congested dilated blood capillaries heavily infiltrating the follicles. This could be attributed to a high level of TSH. This finding was in agreement with that of other studies\cite{63} that reported better vascularization of the gland after methimazole treatment. However, other researchers\cite{64} attributed this vascularization to growth factors and other vasoactive factors produced in the thyroid that was potent angiogenic proteins.

The present work revealed apparent increase in the number of C cells in comparison with the control group. Ultrastructurally, the secretory granules of C cells were numerous, but small, with reduced electron density. Evidence of hyperactivity was found in C cells such as follicular cells. This might be because of the high level of TSH, leading to hyperplasia and hypertrophy of C cells. These findings were in agreement with those of other authors\cite{65, 66}, who have reported that a hyperactive thyroid revealed the presence of enlarged C cells disseminated either in small groups or even singly. These observations might point to the possibility of a relationship between the activity of C cells and the functional state of the thyroid gland. Also, they proposed that the probable mechanisms involved in the changes in C cell with the status of thyroid were consistent with the changes in follicular cells.

It was declared that BPA induced organ toxicity through increasing cellular oxidative stress and decreasing the activity of antioxidants effects\cite{67}. In addition, it was found that exposure to BPA could lead to DNA damage and mutation through severe oxidative stress and reduced antioxidant enzyme activities\cite{68}. The data from many authors revealed that BPA caused noticeable oxidative impact by diminishing the antioxidant enzymes activities and increasing levels of ROS production\cite{69}.

Masson’s Trichrome staining in this work revealed increased greenish colored collagen fibers deposition between severely disrupted thyroid follicles. It was reported that BPA could induce fibroblast hyperplasia in other organs\cite{70}. Injury to the epithelium and basement membranes represents an initial step in the etiology of fibrosis\cite{71}. However, a previous study mentioned that ROS may behave as a second messenger, favoring the activation of transcription factors such as nuclear transcription factor κB. The undesirable consequences of these cellular events include apoptosis, inflammation, fibrosis and excessive production of collagen\cite{72}.

The current work proposed oxidative stress as an adverse effect of BPA. It was assessed by a very strong positive immunoreactivity for iNOS compared with the control. In harmony, some investigators\cite{73} reported that BPA generates reactive oxygen species (ROS) by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation. Other researchers added that formation of ROS arose from the formation of quinine radical, one of the BPA metabolites and from reduced mitochondrial fractions\cite{74}.

It was revealed that the BPA levels, even at very low concentrations, may cause deleterious dangerous effects on human health\cite{75}. Thus, the use of BPA in different plasticizers and other industries should be restricted and the wrong usage of plastic containers should be eluded to lessen the health dangers resulting from exposure to these endocrine disruptors including BPA\cite{76}.

Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. Selenium is a trace element that plays a critical role in several processes for human health. Se is the most powerful antioxidant agent present in the human body\cite{77, 78}. It is probably the next most important mineral (after iodine) affecting thyroid function. Selenium also plays crucial role in the control of THs metabolism. Thyroid is the organ with the highest Se content. Se acts as antioxidant by contrasting the production of the reactive oxygen species that are generated during thyroid hormones biosynthesis.

The present results showed that the group treated with selenium in concomitant with BPA revealed great improvement of the histological appearance compared to the BPA-treated group where most of the thyroid follicles almost had more or less normal architecture. Laboratory results exhibited that the levels of T3, T4 and TSH appeared nearly similar to the control group. The current results concerning Se supplementation which resulted in increasing serum thyroid hormone levels of T4 and T3 in the current study could be elucidated by Rayman\cite{79} who suggested that Se supplementation improved conversion of T4 to T3.

In this study, the correlated findings of thyroid histopathology and thyroid hormone imbalance showed a hypothyroidism by a great decrease in serum T4 and T3 levels. The increased serum TSH levels and the thyroid hyperplasia are consistent with a consequent stimulation of pituitary cells. The pathological outcomes of hypothyroidism were reported to be associated with oxidative stress, increased production of free radicals,
and reduced antioxidative defence\(^{[90]}\). Supplementation with antioxidants could be useful in inhibiting oxidative damage\(^{[89]}\).

Different classes of selenium compounds played a protective role through their antioxidant properties. Se-containing proteins were involved in TH synthesis by protecting the biosynthetic process against the toxicity of free oxygen radicals. Moreover, Se along with other nutritional supplements as iodine and zinc has been recommended for the hypothyroidism treatment, rather than thyroxin administration\(^{[81]}\).

Concerning the effect of selenium, the present study indicated that selenium protected against the histological and immunohistochemical alterations induced by BPA; this comes in agreement with other several investigators who studied the protective effect of selenium against toxicity of different drugs and chemicals\(^{[82]}\). The present results were supported by Prabhu et al.\(^{[83]}\) who demonstrated an inverse relationship of iNOS expression and activity with Se status. They suggested that strategies, such as dietary supplementation of Se, to inhibit NO generation may prove useful in decreasing the risk of chronic inflammatory diseases. In harmony, it was documented that thyroid is one of the organs with the highest content of selenium as it expresses numerous specific selenoproteins of which some are implicated in the metabolism of thyroid hormone and others play an antioxidant defense role. Selenium supplementation appears to potentiate the selenoproteins and others play an antioxidant defense role. Selenium supplementation appears to potentiate the selenoproteins activities, thereby decreasing local inflammatory reactions and improves thyroid morphology\(^{[84]}\).

In harmony with the current findings, it was documented that selenium reduced the oxidative stress, endocrine disorder and apoptosis in the rat testes\(^{[85,86]}\). It was declared that the mechanism of chemoprotection of selenium may be related to its properties as antioxidant besides its ability to hinder with pathways of DNA repair\(^{[87]}\). While Se dependent glutathione peroxidases (GPx) are linked to the gland protection\(^{[77]}\).

The obtained results are in agreement with the finding of\(^{[88]}\) who found that the addition of Se to diet had a partial recovery occurred in body and thyroid gland weights and plasma TSH levels of both mothers and pups. Furthermore, Rasmussen et al.\(^{[89]}\) showed an inverse relationship between the serum Se concentration and the volume of the thyroid gland, reported that Se supplementation found to be significantly associated with thyroid volume regression.

The present study indicated that selenium supplementation had a dramatic protective effect on the structure of thyroid gland. The results obtained in this study correlated with earlier studies concerning the influence of different antioxidants on the same organ. The studies showed a stronger preventive action of selenium on the morphological picture of the thyroid. The current results are in agreement with those of Rasmussen et al.\(^{[89]}\) who found that Se supplementation improved the histopathological features of the thyroid gland. Evidenced by its return to normal aspects in which the phenomena of proliferation and cell death must have been involved. Restoring iodide, after selenium supplementation to diet, reduced the hypertrophy of follicle epithelial cells and increased the colloidal volume. Selenium supplementation improves the ultrasound structure of the thyroid gland\(^{[84]}\).

The results of the previous investigators\(^{[90,91]}\) revealed that treatment of rats with nano-selenium appeared to be counter to the hypothyroid status. This was indicated by restoring serum free T3 and T4 concentrations as well as thyroid antioxidant activity. Thus it succeeded in restoring thyroid integrity by lowering oxidative stress, as the administration of Se nanoparticles caused a significant increase in antioxidant activities as glutathione (GSH) and superoxide dismutase (SOD) and depletion of malondialdehyde (MDA) level.

It is quite clear that administration of selenium to rats inhibited the development of changes observed in thyroids after exposure to BPA. The present result declared cellular thyroid toxicity induced by BPA and avoidance of this damage by administration of selenium. Although few data are available concerning the use of antioxidant against BPA induced thyroid toxicity, the result of the use of selenium against toxicity of one of the environmental pollutants appears to be promising.

In conclusion, Bisphenol A induced variable structural alterations in Follicular and parafollicular cells of thyroid gland. These deleterious effects may be mediated through disruption of cellular organelles that subsequently affects their function. However, selenium supplementation exerted undeniable protective role against these changes.

**RECOMMENDATIONS**

- These adverse effects of BPA on the thyroid gland and overall health may raise the urgent need to pay more attention to this very dangerous substance and to increase community awareness.
- Considering the fact that exposure of this endocrine disruptor is inevitable this necessitates carrying out periodic hormonal essay.
- The usage of BPA in different plasticizers and other industries should be restricted and the wrong handling of plastic containers should be avoided.
- The maintenance of a physiological concentration of selenium (selenostasis) through a balanced diet or, alternatively, via supplementation is a prerequisite not only to prevent thyroid disease but also to maintain overall health.

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CONFLICTS OF INTEREST
There are no conflicts of interest

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دراسة نسيجية وكيميائية للتأثير السمي لمادة البيسفينول على الغدة الدرقية في ذكور الجرذان البيضاء البالغة واحتمال الوقاية بمادة السيلينيوم

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الملخص العربي

دراسة نسيجية وكيميائية للتأثير السمي لمادة البيسفينول على الغدة الدرقية في ذكور الجرذان البيضاء البالغة واحتمال الوقاية بمادة السيلينيوم

المقدمة: البيسفينول (BPA) هو مادة ذات نطاق واسع الاستعمال في جميع أنحاء العالم حيث يتم دمجها في العديد من الصناعات البلاستيكية ومعروف باحداث خلل في الغدد الصماء وقد أثارت العديد من الوكالات التحذيرات ضد الاستخدام المفرط لمثل هذه المادة بسبب آثارها المؤكدة الخطورة على الإنسان. السيلينيوم (Se) معروف بدوره المضاد للأكسدة في الأنسجة الحية.

هدف البحث: الهدف من هذه الدراسة هو تقييم مدى تأثير بيفينول على تركيب ووظيفة الغدة الدرقية والدور الوقائي المحتمل لمادة السيلينيوم.

المواد وطرق البحث: تم تقسيم أربعة وعشرين من ذكور الفئران إلى ثلاث مجموعات. المجموعة الأولى اعتبرت كمجموعة ضابطة ، المجموعة الثانية مجموعة معالجة بمادة BPA وقد شملت الجرذان التي تلقت 50 مجم / كجم / يوم عن طريق الفم بواسطة أنبوب معدي لمدة ثمانية أسابيع، أما المجموعة الثالثة فقد حوت الجرذان التي تلقت بنفس الجرعة واللمرة نفسها بالتزامن مع السيلينيوم بجرعة 0.5 مجم / كجم / يوم. في نهاية التجربة ، تم أخذ عينات من الدم لقياس هرمونات الغدة الدرقية وتحليل النتائج التي تم الحصول عليها إحصائيا .كما تم اخذ عينات من أنسجة الغدة الدرقية وتم تجهيزها للدراسة الهستولوجية والهستوكيميائية مناعية.

النتائج: ظهر الفحص النسيجي لحويصلات الغدة الدرقية في الفئران التي عولجت بمادة BPA علامات انحلال واضحة في شكل انخفاض في المساحة الغروية أو غيابها أو انقطاع لبعض الحويصلات. كما أظهرت حويصلات أخرى علامات فرت النشط التي يتجلّى فيها وجود حويصلات متناهية الصغر وزيادة كثافة للخلايا المبطنة للحويصلات وظهور فجوات، مع زيادة في عدد الليزوزومات. أيضا اظهرت نتائج الدراسة البيستوكيميائية المناعية تفاعل إيجابي ملحوظ للإنزيم المستحث المخلق لأوكسيد النيتروجين (iNOS) في الدم وما صاحب ذلك من انخفاض في T4 و T3 من ناحية أخرى كشفت المجموعة المعالجة BPA + selenium ظهور اطبيعا تقريبا لشكل وتركيب الغدة الدرقية.

الخلاصة: أظهرت الدراسة الحالية أن التعرض ل BPA تسبب في آثار ضارة شديدة على بنية الغدة الدرقية ووظيفتها في حين ان اعطائها متزامنة مع السيلينيوم حافظ إلى حد كبير على بنية الغدة الدرقية.