

A Toxicological, Histological, and Biochemical Study of the Effect of Aroclor 1254 on Thyroid Follicular Cells and the Possible Protective Role of Zinc Sulphate in Adult Male Albino Rats

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Abstract

Introduction: Aroclor 1254 is a commercial mixture of polychlorinated biphenyls used in transformers, capacitors, paints, pesticides and others. The effect of Aroclor 1254 on endocrine system, especially thyroid gland has been a point of research interest.

Aim of the work: The present study was, therefore designed specifically to investigate the possible toxic effect of Aroclor 1254 on thyroid follicular cells of adult male albino rats and to assess the possible protective role of zinc sulphate.

Material and methods: The study was carried out on 45 adult male albino rats that were randomly divided into 3 equal groups. Group I or the control group, which was further, divided into 3 equal subgroups, (5 rats each): received either normal saline or corn oil or zinc sulphate, respectively, orally for 4 weeks. Group II: Aroclor 1254 dissolved in corn oil in a dose of 4mg /kg.b.w/day for 4 weeks. Group III: received Aroclor 1254 dissolved in corn oil in a dose of 4mg /kg.b.w/day with concomitant administration of zn sulphate at a dose of 200mg/kg.b.w/day for 4 weeks. After 28 days, the animals were sacrificed and blood samples were subjected to hormonal assay for T3, T4 and TSH levels. Malondialdehyde (MDA) and reduced glutathione (GSH) were also assessed.

Results: Biochemical assays showed a significant decrease in serum GSH, total antioxidant capacity, T3 and T4 levels in the aroclor treated group than both control and protected group. On contrary, a significant increase in serum MDA and TSH levels was recorded in Aroclor treated group than other two groups. Microscopic examination of thyroid gland follicles in Aroclor 1254 treated rats revealed evident histological changes. Most of the follicles appeared smaller compared to the control group. Excessive vacuolation of the colloid at the interphase with the lining epithelial cells was seen. Moreover, most of the follicles appeared lined with high cuboidal epithelial cells with foci of epithelial hyperplasia. Ultrastructural alterations in follicular cells were detected which appeared as high columnar cells with irregular microvilli. Numerous rough endoplasmic reticulum with extensively dilated cisternae that were filled with flocculent material were demonstrated. On examination of the group III, a marked improvement in the histological features of thyroid follicles was noticed.

Conclusion: These results suggest that PCB induces oxidative stress in rats as well as hypothyroidism; these effects could be reversed by the administration of zinc sulphate. The decrease in T3 and T4 levels induced compensatory effects in the thyroid, visible as hyperactivity of the thyroid epithelia.

Keywords Polychlorinated biphenyls (PCBs), Aroclor 1254, Thyroid, zinc sulphate

Abbreviations

GR: glutathione reductase

PCBs: Polychlorinated biphenyls

T3: triiodothyronine

T4: thyroxin

TAOC: total antioxidant capacity

TSH: thyroid-stimulating hormone

Introduction

Polychlorinated biphenyls (PCBs) are synthetic organic chemicals comprising 209 individual chlorinated biphenyl compounds (known as congeners) exhibiting both dioxin-like and non-dioxin-like toxicity to human and ecological receptors (Giesy and Kannan 1998; Van Den Berg et al., 1998).

Although the commercial production of polychlorinated biphenyls (PCBs) was banned in 1979, The PCBs are considered as persistent organic pollutants (POPs) (Safe, 1994) due to their chemical stability, lipid solubility and their significant bioaccumulation potentials in environmental systems (Kamrin and Ringer, 1994).

Because they are resistant to molecular degradation, PCBs are persistent in the environment, about 1.5 million metric tons are now distributed over the surface of the earth. The most polluted areas of the earth are called "PCB reservoirs" and include the Baltic Sea, Hudson Bay, and the Great Lakes. (Langer, 2008) Sources of PCBs are meat, fish (farmed fish or fish from the Great Lakes have documented high PCB levels), and dairy products (Hanrahan et al., 1999, Pantandin et al., 1999).

PCBs were widely distributed in a variety of industrial purposes; compressors, heat transfer systems, plasticizers, pigments, adhesives, liquid cooled electric motors, fluorescent light ballasts. (Safe, 1994, Erickson, 1997)

Aroclor 1254 is a commercial mixture of polychlorinated biphenyls that is defined as being 54% chlorine by weight (ASTDR, 1987).

PCBs are distributed throughout the entire ecosystem, including soil, air and water. Thus, human exposure to PCBs occurs primarily by way of low-level incidental food contamination. These compounds also have been found at trace levels in marine plant and animal species, fish, mammals, birds, and humans or may be inhaled from contaminated hazardous waste sites (Ross, 2004). Exposure may also occur in workplace environments involved in repair and maintenance of PCB transformers, through accidents and spills, and disposal of PCBs and PCB-contaminated equipments. Intake of PCBs from contaminated soil may occur via ingestion, inhalation or dermal (skin). One of the main sources of these compounds is dietary fish, which accumulate such compounds by direct absorption through the gills, and by exposure to contaminated sediments, consumption of insects and smaller fish (Bush and Kadlec, 1995).

A growing number of studies have found serious health effects from exposure to PCBs. They disrupt normal endocrine functions in human and mammals (Kimbrough, 1995) and exert a diverse spectrum of toxicological and biochemical effects; neurotoxicity (Seegal et al., 1991) body weight loss, immunotoxicity (Sørmoet et al., 2009) and induction of gene expression (El Nemr et al., 2003).

Based on the reports of their harmful effects to wildlife and humans, the PCBs were restricted from use in Egypt. However, due to long environmental half-lives of these compounds, (from ten to twenty

years) (Jones and de Voogt, 1999), the PCBs continue to be detected in sediment samples collected from 34 locations in Lake Qarun particularly near urban areas. Barakat et al., 2002, 2012 (a, b) and Barakat et al., 2013 determined PCBs in surface sediment samples collected from Alexandria Harbor, Lake Manzala and Lake Maryut in Egypt.

PCBs are lipophilic and poorly catabolized and thus, they remain in tissues such as testes, ovaries, adrenals, liver, adipose tissue, skin, and other organs as well as in plasma membranes. (Kimbrough, 1995)

Thyroid gland is an endocrine gland, its follicular and parafollicular cells synthesize and secrete important regulatory hormones such as thyroxine (T4) and triiodothyronine (T3). These hormones are necessary to increase the metabolic activity of most cells, (stimulating growth via induction of DNA translation, which results in greater activity in cell synthesis, oxidative phosphorylation and membrane transport of electrolyte) (Mohamed and El-Gharieb, 2009).

Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's natural antioxidant defense mechanisms, causing damage to macromolecules such as DNA, proteins and lipids. To counteract the damaging effect of ROS, aerobic cells are provided with extensive antioxidant defense mechanisms. These consist mainly of antioxidant enzymes such as glutathione reductase (GR) (Turrens, 2003). PCBs were known to generate transient reactive oxygen species, which disturbs the function of membrane proteins (Tharappel et al., 2002).

The present study was, therefore designed specifically to investigate: (1) the possible toxic effect of Aroclor 1254 on thyroid follicular cells of adult male albino rats, (2) the possible effect of reactive oxygen species (ROS) on the normal function of the thyroid gland and if it is related to PCB toxicity (3) and to assess whether the zinc sulphate simultaneously given with Aroclor 1254 exerts some protective effects on the thyroid tissues.

Materials and methods

Chemicals

Aroclor 1254, corn oil, and Zinc sulphate were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Aroclor 1254 was in the form of 50 mg neat, analytical ampoules. Zinc sulphate was in the form of zinc sulphate concentrate.

Animals and experimental design

A total of 45 specifically pathogen free adult male albino rats (weighting 200-230 g) were included in the present work. All procedures conformed the guidelines for the care and handling of animals and the study protocol was approved by the ethical committee of Alexandria Faculty of Medicine.

The animals were housed under the same laboratory conditions of light and temperature with free access to standard laboratory food and water. They were allowed to acclimatize to the new circumstances

for one week prior to the start of the experiment. The duration of the experiment was 28 days.

The rats were randomly classified into three numerically equal experimental groups (15 animals each) as follows:

1- Group I (control)

This group includes 15 rats and was further divided into 3 numerically equal subgroups:

- Group Ia: Rats of this group received 3ml of distilled water for each rat, orally via orogastric tube.
- Group Ib: Rats of this group received 0.4 ml of corn oil for each rat orally via orogastric tube.
- Group Ic: Rats of this group received Zn sulfate of 200 mg/kg.b.w dissolved in 3ml of distilled water orally via orogastric-tube (Venkataraman et al., 2004).

2- Group II (Aroclor treated- group)

Rats of this group received oral Aroclor 1254 in a dose of 4mg/kg.b.w in 0.4 ml of corn oil daily for 28 days (Buha et al., 2013). LD₅₀ of PCB_s (rat, oral) is 1500mg/kg (Stine and Brown, 2006).

3- Group III (Protected group)

Rats of this group received oral Aroclor 1254 in a dose of 4mg /kg.b.w dissolved in 0.4 ml of corn oil with concomitant administration of zinc sulphate at a dose of 200mg/kg.b.w dissolved in 3 ml of distilled water daily for 28 days (Venkataraman et al., 2004).

Hormonal analysis

Determination of Serum T3, T4 and TSH

At the end of the experimental period (28 days), blood samples were collected from rat carotid arteries. Centrifugation was done and serum was kept at -20 °C for further analysis of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) levels by ELISA (Kuriyama et al., 2007). Then, rats were sacrificed by decapitation under light ether anesthesia.

Antioxidant enzymes and lipid peroxidation

For determination of markers of oxidative stress, blood samples were collected without using any anticoagulant and then the blood was allowed to clot for 30 minutes at 25°C. The blood was centrifuged at 4000 rpm for 15 minutes at 4°C. A Pipette was used to separate the top yellow serum layer without disturbing the white buffy layer.

Assessment of total antioxidant capacity (TAOC)

To determine the effect of aroclor 1254 on oxidative stress and consequently the potential beneficial effect of zinc sulphate, total antioxidant capacity was measured. The assay was determined via the method of Koracevic et al., (2001). TAOC determination is based on the development of a colored product when hydrogen peroxide is added. The values are expressed as mM/L.

Reduced glutathione

Reduced glutathione (GSH) content was determined via the method of Miwa et al., (1989). GSH determination is based on the development of a yellow color when 5,5' dithio (2-nitro benzoic acid) (DTNB) is added to compounds containing sulfhydryl groups. The values are expressed as mg/dL.

Malondialdehyde

Malondialdehyde (MDA) was used as a biochemical marker for lipid peroxidation and was measured by the method described by Ohkawa et al., (1979) using thiobarbituric acid (TBA). MDA was measured by reading the absorbance at 532 nm and expressed as nmol/ml.

Histological study

The thyroid glands were immediately removed after scarification and further processed for:

Light microscopic study

One lobe of the thyroid gland was fixed in 10% formal saline and processed to get 6µm thick, paraffin sections for light microscopic examination using Haematoxylin and Eosin (H&E) stain (Drury and Wallington, 1980).

Electron microscopic study

The other lobe of the thyroid gland was cut into small pieces about 2-3 mm³, then immersed immediately in 3% phosphate buffered glutaraldehyde (PH 7.4) at 4°C. The specimens were further processed for ultrastructure examination by Joel-100 CX transmission electron microscope (Trevor and Graham, 1995) at the Electron Microscopic Unit, Faculty of Science, Alexandria University.

Histomorphometric study

The diameters of 20 randomly selected, thyroid follicles were measured from H&E-stained sections of the thyroid gland of each group of rats at magnification of 100 using the Image Analyzer (Olympus BX41TF, Tokyo, Japan) at the Cell Biology Department, Medical Research Institute, Alexandria University. Such data were subjected to biostatistical analysis.

Data were subjected to statistical analysis using statistical package for social sciences (SPSS) version 20 and the following were calculated: Range (minimum and maximum), Means and Standard deviations. For normally distributed data, comparison between the three studied groups was analyzed using F-test (ANOVA) and Post Hoc test (Scheffe). Significance test results were quoted as two-tailed probabilities. P-value less than 0.001 was considered statistically significant (Leslie et al., 1991).

Results

Biochemical Results

In the present work, no statistical difference was found among the three subgroups of the control group. A significant decrease in the levels of T3 and T4 has been recorded after 4 weeks period of Aroclor 1254 administration when compared with the control groups and the protected group (Tables 1 and 2). On the contrary, the serum levels of TSH were significantly

increased in the Aroclor treated group (Table 3). In addition, the level of serum GSH and the total antioxidant capacity were decreased in the Aroclor treated group (Tables 4 and 5). Table 6 demonstrates a significant increase in serum MDA levels in the Aroclor treated group when compared with the other two groups.

Histological results

Histomorphometric study

The present study revealed that there was a significant decrease in the mean value of the diameters of thyroid follicles in group II (Aroclor 1254 treated group) as compared with the control group ($P < 0.001$), as well, with group III ($P < 0.001$). Furthermore, the mean value of the diameters of thyroid follicles in group III showed also a significant decrease as compared with group I (P -value < 0.001) (Table 7).

Light Microscopic results

Group I (Control group)

Histological examination of control group rats showed classical appearance of thyroid follicles of different sizes but mostly large in size. Homogenous eosinophilic colloid was seen filling the lumen of the follicles. The follicles were seen lined with a single layer of low cuboidal epithelial cells with vesicular nuclei. No histological changes were noted among the three subgroups of the control rats (Figures 1a and 1b).

Group II (Aroclor treated group)

Examination of thyroid gland follicles of aroclor 1254 treated rats revealed evident histological changes. Most of the follicles appeared small in size and crowded, compared to the control group (Figure 2). Excessive vacuolation of the colloid at the interphase with the lining epithelial cells was seen. Moreover, most of the follicles appeared lined with high cuboidal epithelial cells. Foci of epithelial stratification were noticed where some follicles appear lined by two layers cells. Cytoplasmic vacuolation of many follicular cells was evident. As for the nuclei, mostly appeared as vesicular nuclei in most of the lining cells. Some other nuclei appeared with nucleolar margination or with irregular outline (Figures 3a and 3b).

Group III (Protected group)

On examination of this group of rats, marked improvement in the histological features of thyroid follicles was noticed. Thyroid follicles appeared of variable sizes but mostly larger in comparison to group II rats, with almost homogeneously stained eosinophilic colloid. In addition, most of the follicles appeared lined with low cuboidal epithelial cells that showed minimal cytoplasmic vacuolation. Some other follicles appeared lined by flat squamous cells. As regards the nuclei, most of them appeared vesicular, while some other nuclei appeared dark (Figures 4a and 4b).

Electron microscopic results

Group I (Control group)

Histological examination of control group rats (the three subgroups) revealed classical appearance of thyroid follicular cells that appeared as a single layer of cuboidal cells lining thyroid follicles. The cells appeared resting on a prominent basal lamina with prominent microvilli on the luminal surfaces. Tight junctions along the upper part of lateral cell borders were also evident. The cytoplasm showed many well organized, tubular profiles of rough endoplasmic reticulum with apparent lumen filled with flocculent material. Well developed Golgi complexes were also seen in the upper parts of the cells. Mitochondria with transverse cristae appeared scattered throughout the cytoplasm. The nuclei appeared with normal chromatin pattern and were basally situated (Figures 5a and 5b).

Group II (Aroclor treated group)

Examination of thyroid gland follicles of aroclor 1254 treated rats revealed evident histological changes, where many follicular cells appeared as high columnar cells with prominent, long and irregular microvilli on the luminal surfaces (Figures 6a and 6b). Some other cells appeared as high cuboidal cells with short and thick microvilli (Figure 7). Papillary projections extending from luminal surface of some follicular cells were also encountered, together with few microvilli (Figure 8). Cytoplasm of most follicular cells showed many profiles of rough endoplasmic reticulum with extensively dilated cisternae filled with flocculent material. As for Golgi complexes, they appeared more prominent than in the control group with relatively dilated cisternal profiles. Some mitochondria appeared vacuolated with disrupted cristae as well (Figures 6 and 7). Many colloid droplets were also seen in the upper parts of some cells (Figure 7). Other cells depicted many lysosomes (Figures 6b and 9). As regards the nuclei some showed dilated perinuclear cisternae (Figure 6a). Some other nuclei appeared dark, shrunken or with irregular outline. The nuclei revealed abnormal chromatin pattern in the form of nucleolar margination and peripheral chromatin clumping (Figures 6b, 7, and 8).

Group III (Protected group)

On examination of this group of rats, marked improvement in the histological features of thyroid follicular cells was noticed. Most of the cells appeared as low columnar or cuboidal cells with prominent short microvilli on the luminal cell surfaces compared to the rats of group II. Also, rough endoplasmic reticulum was less numerous and appeared with moderately dilated tubular profiles. Golgi complexes appeared with almost control pattern. As regards the nuclei, some appeared with irregular outline and nucleolar margination (Figure 10). Some other nuclei revealed almost normal chromatin pattern (Figure 11).

Table (1): Serum levels of T3 in the Aroclor1254-treated group compared with the control and the protected groups

Parameter	Group	Range	Mean \pm SD	F	P	Pair wise comparison
T3 (Pg/ml)	I:Control	1.2 – 1.6	1.4 \pm 0.16	306.167	<0.001*	I-II*
	II: Aroclor treated	0.1 – 0.16	0.12 \pm 0.02			I-III*
	III: Protected	0.18 – 0.9	0.53 \pm 0.19			II-III*

F: F test (ANOVA); Pair wise comparison was done using Post Hoc Test (Scheffe); *: Statistically significant at $p \leq 0.001$

Table (2): Serum levels of T4 in the Aroclor1254-treated group compared with the control and the protected group

Parameter	Group	Range	Mean \pm SD	F	P	Pair wise comparison
T4(Pg/ml)	I:Control	15– 22	17.73 \pm 1.71	39.13	<0.001*	I-II*
	II: Aroclor treated	6 – 13	10.33 \pm 1.87			I-III*
	III: Protected	11 – 21	15.33 \pm 3.15			II-III*

F: F test (ANOVA); Pair wise comparison was done using Post Hoc Test (Scheffe); *: Statistically significant at $p \leq 0.001$

Table (3): Serum levels of TSH in Aroclor1254-treated group compared with the control and the protected group

Parameter	Group	Range	Mean \pm SD	F	P	Pair wise comparison
TSH (μ IU/ml)	I:Control	0.10 – 0.40	0.23 \pm 0.10	188.655*	<0.001*	I-II*
	II: Aroclor treated	2.70- 3.20	2.91 \pm 0.15			I-III*
	III: Protected	0.70- 2.60	1.74 \pm 0.63			II-III*

F: F test (ANOVA); Pair wise comparison was done using Post Hoc Test (Scheffe); *: Statistically significant at $p \leq 0.001$

Table (4): Comparison between the three studied groups according to GSH

Parameter	Group	Range	Mean \pm SD	F	P	Pair wise comparison
GSH (mg/dL)	I:Control	1.39 – 2.8	2.19 \pm 0.41	48.864	<0.001*	I-II*
	II: Aroclor treated	0.78–1.31	0.99 \pm 0.15			I-III*
	III: Protected	0.99 -2.39	1.73 \pm 0.38			II-III*

F: F test (ANOVA); Pair wise comparison was done using Post Hoc Test (Scheffe); *: Statistically significant at $p \leq 0.001$

Table (5): Comparison between the three studied groups according to TAOC level

Parameter	Group	Range	Mean \pm SD	F	P	Pair wise comparison
TAC (mM/L)	I:Control	1.34 – 2.9	1.83 \pm 0.46	62.83	<0.001*	I-II*
	II: Aroclor treated	0.29 -0.83	0.57 \pm 0.22			I-III*
	III: Protected	0.89 -1.43	1.11 \pm 0.17			II-III*

F: F test (ANOVA); Pair wise comparison was done using Post Hoc Test (Scheffe); *: Statistically significant at $p \leq 0.001$

Table (6): Comparison between the three studied groups according to MDA level

Parameter	Group	Range	Mean \pm SD	F	P	Pair wise comparison
MDA (nmol/ml)	I:Control	3.69 – 5.2	4.49 \pm 0.57	120.35	<0.001*	I-II*
	II: Aroclor treated	7.12 -9.55	8.43 \pm 0.77			I-III*
	III: Protected	5.2 – 7.62	6.35 \pm 0.74			II-III*

F: F test (ANOVA); Pair wise comparison was done using Post Hoc Test (Scheffe); *: Statistically significant at $p \leq 0.001$

Table (7): Comparison between the different studied groups according to diameter of thyroid follicles in pixels

Diameter of thyroid follicles (Pixels)	I: Control	II: Aroclor treated	III: Protected	F	p
Min. – Max.	391.06 – 751.80	158.35 – 299.39	158.35 – 480.0	80.029*	<0.001*
Mean \pm SD	535.88 \pm 107.43	227.10 \pm 40.53	304.93 \pm 78.43		
Median	502.06	233.75	297.23		
p₁		<0.001*	<0.001*		
p₂				0.013*	

F: F test (ANOVA); p_1 : p value for Post Hoc test (Scheffe) for comparing between group I and each other group; p_2 : p value for Post Hoc test (Scheffe) for comparing between group II and group III; *: Statistically significant at $p \leq 0.001$

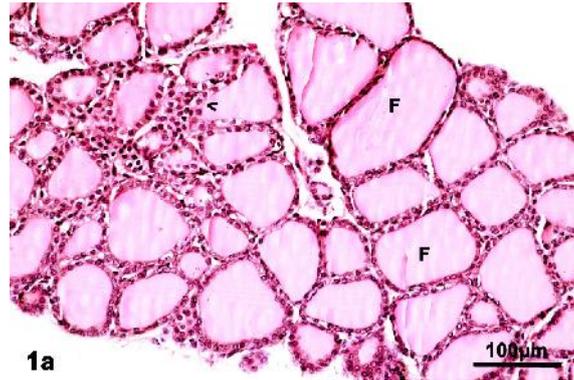


Figure 1a: A photomicrograph of thyroid gland follicles of a control group (group I) rat, showing thyroid follicles (F) lined by simple cuboidal epithelium with vesicular nuclei (<). Most of the follicles appear large in size.

H&E, MicMag x200

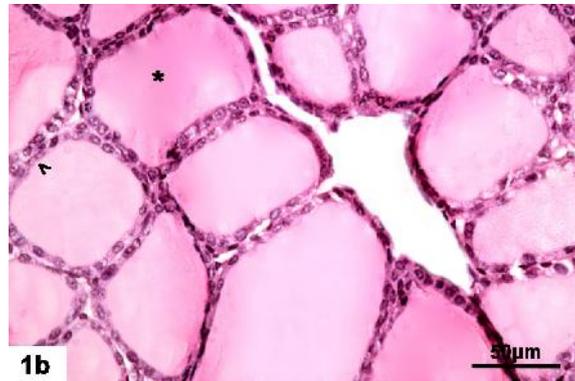


Figure 1b: A photomicrograph of thyroid gland follicles of a control group (group I) rat, showing thyroid follicles lined by simple cuboidal epithelium with vesicular nuclei (<). Homogenous eosinophilic colloid (*) is seen filling the lumen of the follicles. Most of the follicles appear large in size.

H&E, MicMag x400

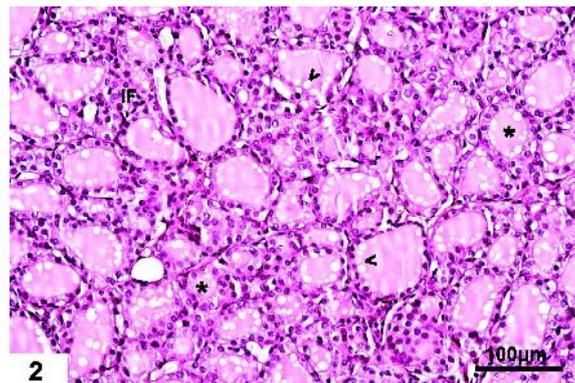
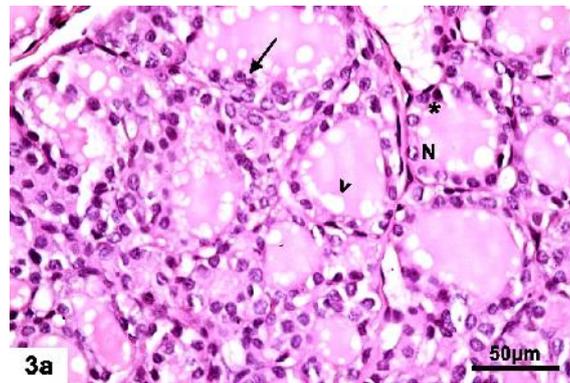
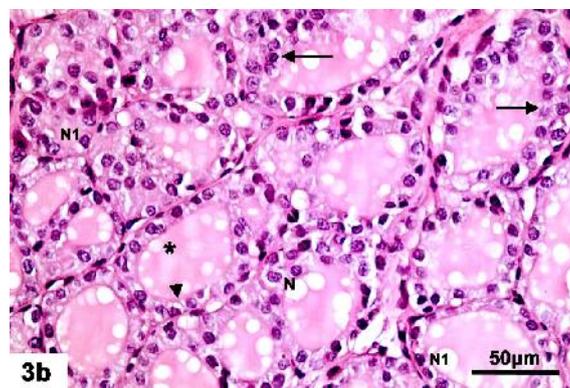


Figure 2: A photomicrograph of thyroid gland follicles of aroclor 1254 treated group (group II) rats. Most of the follicles (*) appear smaller in size in comparison to the control group I. Extensive vacuolation (<) of the colloid is also noticed. (IF); interfollicular cells.

H&E, MicMag x200



Figures 3a: A Photomicrograph of thyroid gland follicles of aroclor 1254 treated group (Group II), showing foci of epithelial stratification (\rightarrow) in some of the follicles. Other follicles appear lined by high cuboidal cells with oval nuclei (*). Evident vacuolation (\lessdot) of the colloid is seen in most of the follicles. Some nuclei (N) appear with abnormal chromatin pattern.
H&E, MicMag x 400



Figures 3b: A Photomicrograph of thyroid gland follicles of aroclor 1254 treated group (Group II), showing foci of epithelial stratification (\rightarrow) in some of the follicles. Other follicles appear lined by high cuboidal cells with oval nuclei (*). Evident vacuolation (\lessdot) of the colloid is seen in most of the follicles. Some nuclei (N) appear with abnormal chromatin pattern; cytoplasmic vacuolation (\blacktriangleright) of most of the follicular cells is also noticed. Other nuclei (N1) appear dark and shrunken.
H&E, MicMag x400

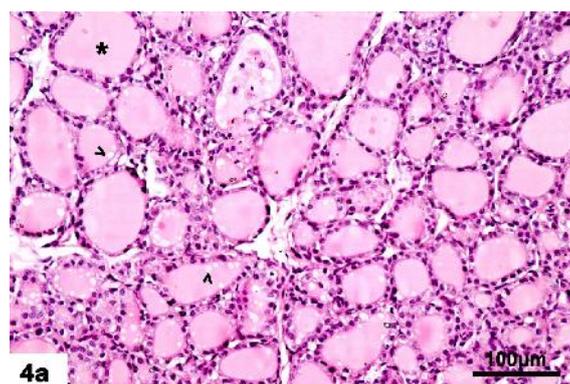


Figure 4a: A photomicrograph of thyroid gland follicles of the protected group (group III), showing many large follicles (astrix). Minimal vacuolation (\lessdot) of the colloid.
H&E, MicMag x200

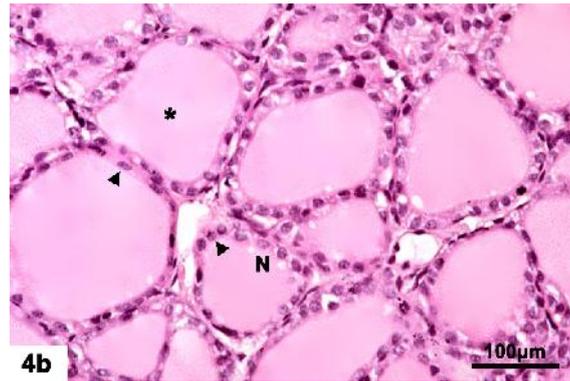


Figure 4b: A photomicrograph of thyroid gland follicles of the protected group (group III), showing many large follicles (astrix). Minimal vacuolation (<) of the colloid; most of the follicles appear filled with homogenous colloid (*). Notice most of the epithelial cells lining the follicles are low cuboidal cells with minimal cytoplasmic vacuolation (▶). Nuclei (N) appear as vesicular nuclei with almost normal chromatin pattern.

H&E, Mic Mag x400

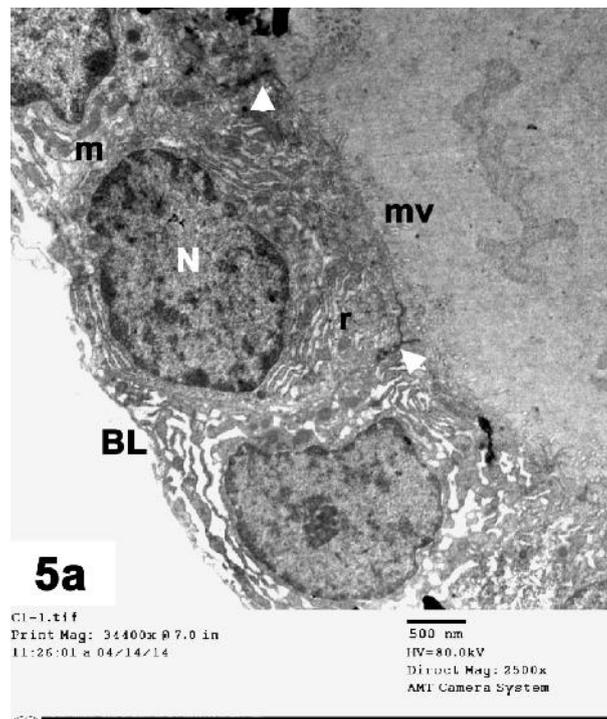


Figure 5a: Electron micrograph of thyroid follicular cells of the control group (Group I), appearing with a classical appearance of a single layer of cuboidal cells. Prominent microvillous border (mv) is seen on the luminal cell surface. Tight junctions (▶) are evident along the upper part of lateral cell borders. Tubular profiles of rough endoplasmic reticulum (r) with apparent lumen are also noticed. Mitochondria (m) with transverse cristae are also apparent. Vesicular nuclei (N) with normal chromatin pattern are seen within the basal parts of the cells. (BL); basal lamina. x2500

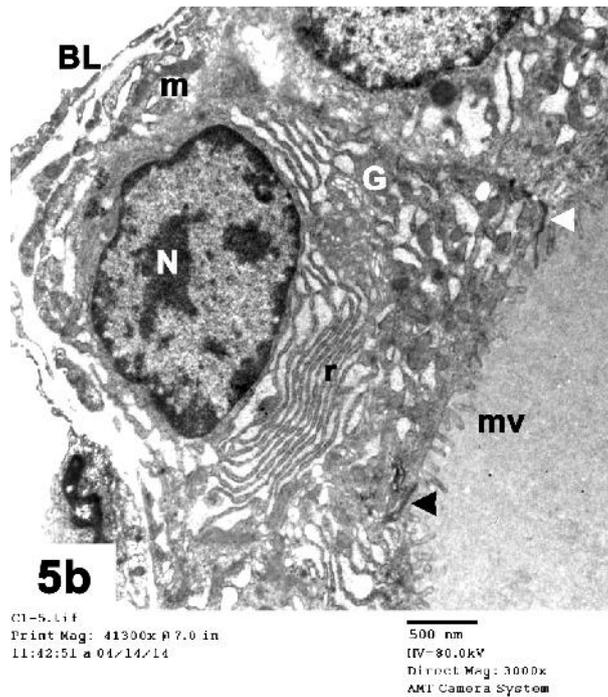


Figure 5b: An Electron micrograph of thyroid follicular cells of the control group (Group I), appearing with a classical appearance of a single layer of cuboidal cells. Prominent microvillous border (mv) is seen on the luminal cell surface. Tight junctions (▶) are evident along the upper part of lateral cell borders. Tubular profiles of rough endoplasmic reticulum (r) with apparent lumen are also noticed. Mitochondria (m) with transverse cristae are also apparent. Vesicular nuclei (N) with normal chromatin pattern are seen within the basal parts of the cells. A small Golgi complex (G) is apparent. (BL); basal lamina. x3000

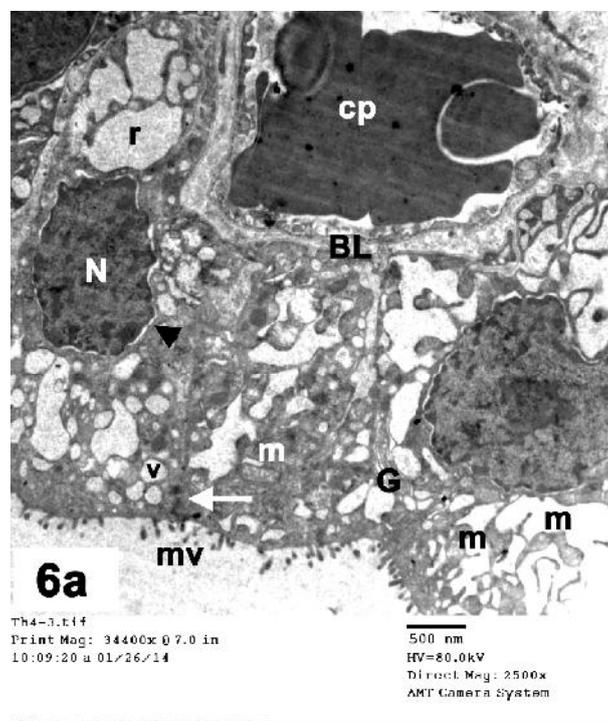


Figure 6a: An Electron micrograph of thyroid follicular cells of aroclor 1254 treated group (group II), showing tall columnar follicular cells with prominent and long microvilli (mv). Rough endoplasmic reticulum (r) appears with markedly dilated cisternal profiles. Prominent Golgi complexes (G) are also seen in upper parts of the cells. Notice some mitochondria (m) appear vacuolated with disrupted cristae. Some vesicles (v) are apparent. Notice evident tight junctions (→). Some nuclei (N) appear with dilated perinuclear cisternae (▶). x2500

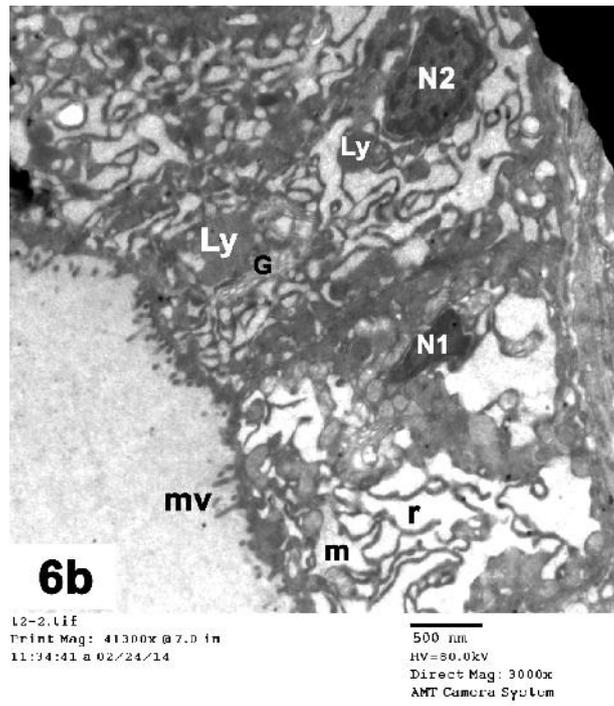


Figure 6b: An Electron micrograph of thyroid follicular cells of aroclor 1254 treated group (group II), showing tall columnar follicular cells with prominent and long microvilli (mv). Rough endoplasmic reticulum (r) appears with markedly dilated cisternal profiles. Prominent Golgi complexes (G) are also seen in upper parts of the cells. Notice some mitochondria (m) appear vacuolated with disrupted cristea. Abundant lysosomes (Ly) are seen. One nucleus (N1) appears dark and shrunken. Another nucleus (N2) reveals abnormal chromatin pattern and irregular outline. x3000

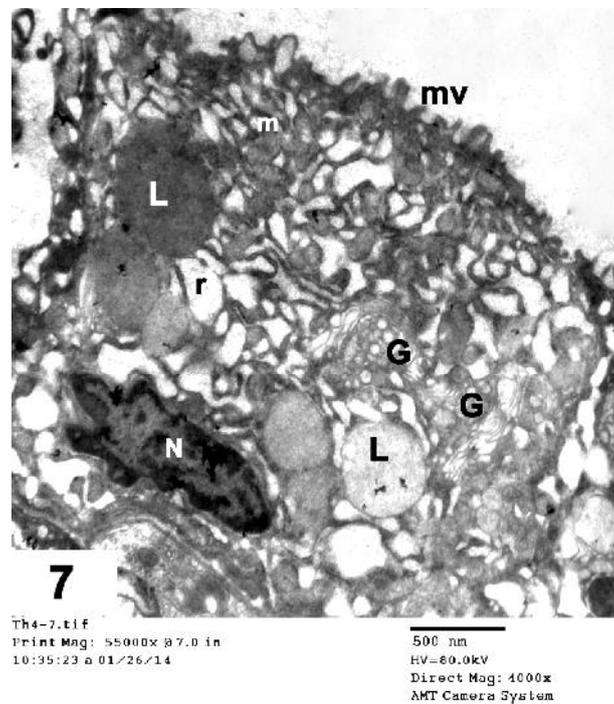


Figure 7: An electron micrograph of a thyroid follicular cell of aroclor 1254 treated group (group II), showing high cuboidal follicular cell with thick, short and irregular appearance of the microvilli (mv). Many colloid droplets (L) are noticed. Prominent Golgi complexes (G) are also seen. Notice mitochondria (m) with disrupted cristae. The nucleus (N) appears with irregular outline and peripheral chromatin clumping. Mic Mag x4000

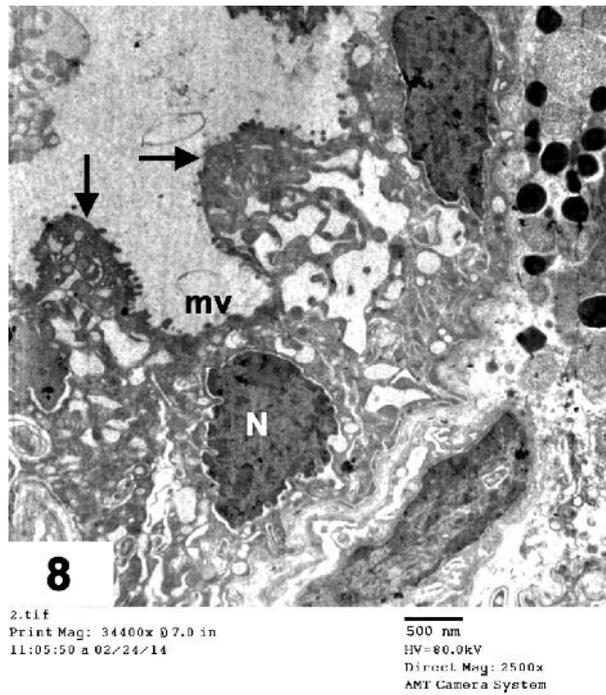


Figure 8: An electron micrograph of thyroid follicular cells of aroclor 1254 treated group (Group II), revealing papillary projections (→). The luminal cell surface appears with few and disrupted microvilli (mv). Notice the nucleus (N) with irregular outline and nucleolar margination. Mic Mag x2500

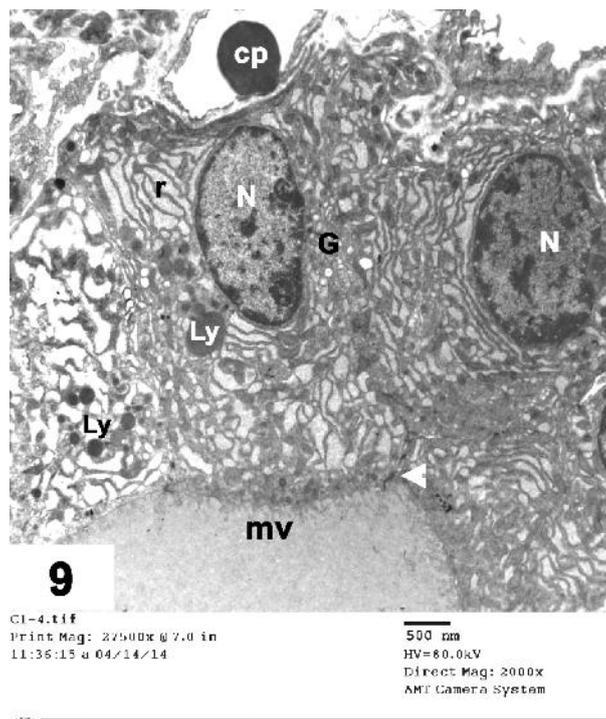


Figure 9: An electron micrograph of thyroid follicular cells of aroclor 1254 treated group (Group II), showing high columnar cells with oval nuclei (N). Multiple lysosomes (Ly) are noticed. Moderately dilated rER (r) is seen. Notice intact tight junctions (▶). (mv); microvilli, (G); Golgi complex, (cp); blood capillary. Mic Mag x2000

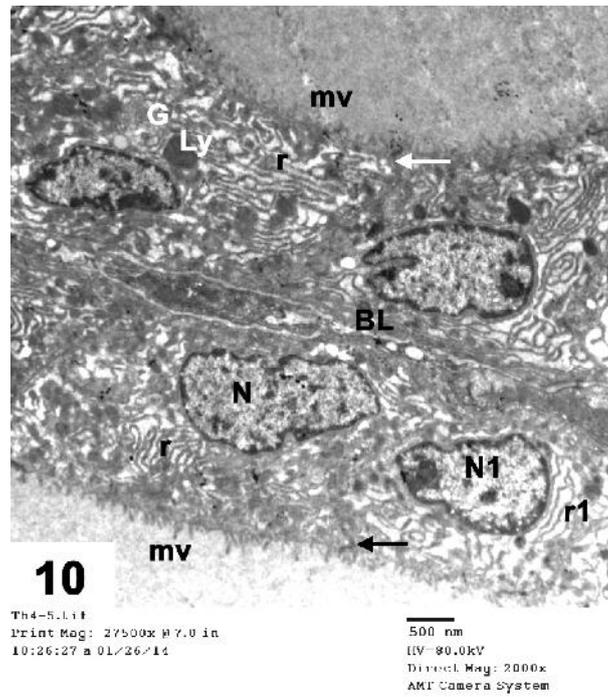


Figure 10: An electron micrograph of thyroid follicular cells of protected group (Group III), showing cuboidal thyroid follicular cells with apparent microvillous border (mv). Some tubular profiles of the rough endoplasmic reticulum (r) are seen with almost control pattern. Some other profiles (r1) of rER appear moderately dilated. Notice apparent tight junctions (→) are seen along the upper part of lateral cell borders. One nucleus (N) appears with almost normal chromatin pattern. Another nucleus (N1) shows nucleolar margination and an irregular outline. (Ly); lysosomes, (BL); basal lamina. Mic Mag x2000

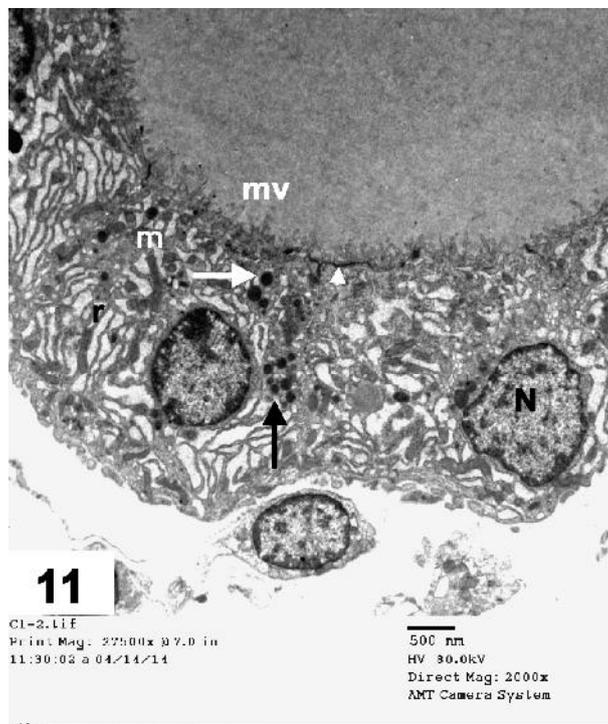


Figure 11: An electron micrograph of thyroid follicular cells of protected group (Group III), showing low columnar follicular cells with prominent microvillous border (mv) and tight junctions (▶). Tubular profiles of rER (r) appear moderately dilated. Some mitochondria (m) appear with transverse cristae. Notice some lysosomes (→) are apparent. Nucleus (N) is seen with almost normal chromatin pattern. Mic Mag x2000

Discussion

Over the last two decades, there has been increasing scientific concern and public debate regarding the

adverse effects of chemical pollutants present in the environment that can interfere with the normal

functioning of the endocrine system (Endocrine disruptors) (Verma and Rana, 2009).

Thyroid hormones are essential for normal body metabolism, growth and development including reproduction and maturation. (Khan et al., 1999) Thyroid hormone homeostasis appears to be the target of numerous natural, as well as man-made chemicals in all vertebrates (Leatherland, 1994). In mammals, thyroid glandular activity is commonly determined by thyroid hormone secretion rate (Lu and Anderson, 1994). Nevertheless, histological examination of the thyroid gland can be a more sensitive early indicator of the glandular activity than serum T3 and T4 (Saeed and Hansen, 1997). As such, the present study was assigned to assess the alteration in thyroid homeostasis that might occur following exposure to Aroclor 1254. The emphasis so far has been on thyroid follicular cell histological features and hormone levels T3 and T4 as well as TSH. In addition, the possible effect of reactive oxygen species (ROS) on the normal function of the thyroid gland was investigated and the possible protective role of zinc sulphate when given simultaneously with Aroclor 1254.

The rats in group II that received Aroclor 1254 for 28 days demonstrated significant decrease in the serum levels of T3 and T4 along with the evident increase in TSH levels, providing evidence that PCBs disrupted the hypothalamic-pituitary-thyroid axis. This was further supported by light microscopic results that showed histological features of hypothyroidism. They were in the form of many small sized follicles, lined with high cuboidal epithelial cells. This was further verified by histomorphometric study. Foci of epithelial hyperplasia were also noticed in some follicles, along with excessive vacuolation of the colloid at the interphase. On the other hand, some histological findings were related to direct effect of Aroclor 1254 through induction of oxidative stress, where many follicular cells appeared with cytoplasmic vacuolation. As regards the nuclei, some nuclei appeared vesicular while some others appeared irregular with abnormal chromatin pattern.

Ultra structurally, the most prominent alterations of follicular cells of rats exposed to Aroclor 1254 for 4 weeks were; numerous rough endoplasmic reticulum with extensively dilated cisternae that were filled flocculent material. Also, prominent Golgi complexes with relatively dilated cisternal profiles. Some mitochondria appeared with disrupted cristae. Many colloid filled droplets were also seen in the upper parts of the cells together with some lysosomes. Some follicular cells showed high columnar cells with few, short and irregular microvilli on the luminal surfaces. Furthermore, some of the nuclei appeared dark and shrunken while the others showed dilated perinuclear cisternae.

The mechanism by which PCBs might produce an increased thyroid volume and increased prevalence

of thyroid disorders remains unexplained. However, disturbed levels of thyroid hormones and TSH could be attributed to several mechanisms. One of the several hypotheses proposed to explain the mechanism by which Aroclor induced thyroid toxicity was increased biotransformation, deactivation and biliary excretion of T4 (Bastomsky, 1974, Bastomsky et al., 1976, Collins et al., 1977). More specifically, the reduction in plasma T4 levels could be explained by induction of the hepatic microsomal enzyme UDP-glucuronosyl-transferase (UDP-GT), which is a phase II enzyme. This enzyme catalyzes the glucuronidation of T4 and thus enhances the biliary excretion of T4-glucuronide (Barter and Klaassen, 1992, Barter and Klaassen, 1994).

Another explanation that has been put forward was that induction of microsomal enzymes that might lead to formation of different metabolites. In turn, such metabolites affect thyroid hormones metabolism. For example, PCBs which are metabolized to hydroxylated PCBs in the liver, possibly by P450 enzymes (Liu et al., 2006) have been shown to interfere with thyroid hormone transport and inhibit thyroid hormone sulfation (Wang and James, 2006). Moreover, hydroxylated PCBs which are structurally similar to thyroid hormones, can bind to plasma TH-transporter transthyretin (TTR) or displace TH from their serum binding proteins and thereby cause the increased T4 excretion and decrease in plasma levels of T4 (Brouwer et al. 1988, Lans et al., 1993; Waller and Mc Kinney 1994; Martin and Klaassen 2010; Zoeller 2010).

Recent in vitro studies have reported that PCBs interfere with thyroid function at receptor or DNA level as PCBs have T3-like properties (Kitamura et al., 2005). At post-receptor level, PCBs inhibited TSH-stimulated adenylate cyclase activity and the cAMP production (Santini et al., 2003). Furthermore, Aroclor 1254 significantly increased expression of TH responsive genes in the fetal cortex in the study conducted by Gauger et al., (2004).

The net result of the previously mentioned disturbance in thyroid hormones metabolism was decreased serum levels of T3 and T4, together with feedback increased serum level TSH as recorded in the present study. These findings agree with those of Anbalagan et al., (2003) and Khan and Hansen (2003)

TSH secretion by the pituitary is known to be inversely related to thyroid hormone levels that serve as the basis for feedback control of circulating thyroid hormones. The synthesis of TSH protein subunits and its secretion are strongly suppressed by T3 but are up regulated in the absence of T3 (Wade et al., 2002).

In such a context, the histological changes seen in group II rats, most probably reflect compensatory increased activity of the thyroid follicular cells in response to elevated TSH level. It is likely to be an attempt of the cell to increase its activity by increasing the cell volume and surface area. This

was achieved through the epithelial hyperplasia in some of the thyroid follicles, as well as, the small sized thyroid follicles seen by the light microscopic examination. Also, increased endocytotic activity of the thyroid follicular cells was reflected by excessive colloidal vacuolation.

Light microscopic results were further supported by the electron microscopic findings. Increased follicular cell activity was evident by presence of high columnar cells lining some of the follicles and some revealed papillary projections. In addition, excessive dilated cisternal profiles of rER were evident, thus reflecting increased synthetic activity of the follicular cells in response to increased hormonal stimulation, where thyroglobulin is synthesized and glycosylated in rER. In accordance, there was increased secretory activity of the cells as indicated by increased cisternal profile of Golgi complexes that also appeared dilated, where Golgi complex is site for completing process of glycosylation of thyroglobulin. In addition, the modified protein is then packaged in trans Golgi network into apical secretory vesicles. As for increased lysosomes, they reflected increased activity of cells or exhaustion of the mechanism whereby the cell rids itself of metabolic debris, or from a disturbance in the enzymatic process of thyroglobulin degradation.

Tall microvilli seen projecting from the luminal surface of some cells was consistent with the histological features of increased cell activity. Thyroid peroxidase enzyme involved in oxidation of iodide, is an integral membrane protein of the microvilli membrane. Thus, providing excessive peroxidase enzyme needed for increased iodination. Nevertheless, increased length of microvilli provided more surface area available for hormone transport. Also, increased lipid droplets in some follicular cells, further confirmed increased endocytotic activity of the cell.

Oxidative stress results from an imbalance between the excessive formation of reactive oxygen species (ROS) and limited antioxidant defenses (Turrens, 2003). The elevated levels of ROS result in cellular damage by peroxidation of membrane lipids, sulfhydryl enzyme activation, protein cross linking and DNA breakdown (Srinivasan et al., 2002). The mitochondrial membrane has been reported to undergo permeability changes following enhanced lipid peroxidation and glutathione depletion (Chance et al., 1979). Accordingly, recent experimental studies have reported that Polychlorinated biphenyls, especially higher chlorinated compounds, may selectively induce cytochrome P450s as a possible source of reactive oxygen species (Ludewiget al., 2000, Schlezinger et al., 2000, Mariussen et al., 2002).

The results of the present study showed that there was significant decrease in the level of both serum GSH and total antioxidant capacity in the Aroclor treated group. Glutathione is the primary endogenous antioxidant that neutralizes reactive

oxygen and nitrogen species from the cell through scavenging. These results are in agreement with the finding reported by Sekaran et al., (2012).

Levels of MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, have been considered as an important indicator of lipid peroxidation (Kalender et al., 2004). Lipid peroxidation has been suggested as one of the molecular mechanisms involved in PCBs induced toxicity. It has been used as a measure of the chemicals induced oxidative stresses (Kehrer, 1993). In the present study, MDA level in Aroclor 1254 treated rats was significantly ($p < 0.001^*$) higher than that in both the control group and the protected group. Such biochemical result was verified histologically by both light and electron microscopic study; some follicular cells showed cytoplasmic vacuoles and degenerated mitochondria with disrupted cristae. In addition, some nuclei appeared dark and shrunken with nucleolar margination. Also, some follicular cells revealed decreased microvilli or short and thick microvilli.

Aly et al., (2009) in a recent study attributed the potential toxic effect of aroclor to alteration in the antioxidant defense mechanism. They studied the effect of Aroclor 1254 on lipid peroxidation and on the some component of the antioxidant defense mechanism. They recorded that levels of hydrogen peroxide (H_2O_2) generation and lipid peroxidation (LPO) were significantly increased in mitochondria in a dose-related pattern.

Recent findings also suggest that PCBs causes decrease activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) and increases H_2O_2 generation and LPO levels (Ross, 2004).

Zinc sulphate is a homeostatically regulated essential mineral. It is a component of numerous metalloenzymes, and it is important for cell growth and replication, osteogenesis, and immunity (Kaji, 2001). Zinc has close interrelationships with endocrine system and is essential for reproductive and thyroid function (Vallee and Falchuk, 1993). Zinc deficiency was shown to impair the metabolism of thyroid hormones (Kralik et al., 1996).

The protective effect of zinc sulphate observed in the present work could be ascribed to the antioxidant effects displayed by it. There has been appreciable evidence arising from different in vitro and in vivo models that indicate that zinc play an important role in suppression of free radicals. This is achieved via its effect on metallothionin synthesis and as inhibitor of NADPH-dependent lipid peroxidation (Prasad, 1997), as well as, preventing lipid peroxidation by inhibition of glutathione depletion (Gibbs et al., 1985). In the present work, simultaneous zinc sulphate and Aroclor 1254 administration for 28 days (group III) was shown to ameliorate the thyroid follicular lesions induced by Aroclor. The thyroid follicles appeared larger in

comparison to group II rats. This was further verified by histomorphometric study that revealed that there was a significant increase in the mean diameters of thyroid follicles in this group as compared to group II. The follicles were lined with low cuboidal epithelial cells or even flat squamous cells as evidenced by both light and electron microscopic findings. Moreover, most of the follicles appeared with homogenous colloid, where limited colloidal vacuolation was depicted. Also, follicular cells appeared with minimal cytoplasmic vacuolation. Ultrastructurally, rER appeared with moderately dilated tubules and fewer Golgi complex profiles were noticed. As regards the nuclei, some appeared with normal chromatin pattern, but still some other nuclei depicted irregular outline and abnormal chromatin pattern. In accordance with the histological findings, biochemical results showed that zinc supplementation restored the levels of T₃, T₄, and TSH in Aroclor 1254 exposed rats.

In accordance with the present results, Venkataraman et al., (2004) in an experimental study on rat prostate exposed to PCBs, recorded restoration of the serum T₃, T₄ and TSH levels with administration of zinc sulphate. They suggested that PCBs induces oxidative stress in rat ventral prostate by decreasing the levels of antioxidant enzymes and these effects could be reversed by the administration of zinc sulphate.

The protective effects of zinc sulphate have been previously addressed in various models of oxidative stress-induced cell damage such as protein malnutrition (Sidhu et al., 2005) and liver and kidney injury induced by organophosphorus insecticides (Mansour and Mossa, 2010).

From the present results, it could be concluded that the thyroid gland is a target for Aroclor 1254 toxicity as indicated by disturbances of T₃, T₄ and TSH hormones levels as well as its histological changes. This raises attention to the emerging latent problem of using PCBs. The risk of thyroid toxicity might occur in humans even with the small dose exposure. Furthermore, the results demonstrate the efficacy of zinc sulphate in ameliorating the thyroid lesions. Regulation should be considered concerning the handling and the public education about the potential health problems related to PCBs.

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الملخص العربي

دراسة سمية وهستولوجية وبيوكيميائية لتأثير أروكلور ١٢٥٤ على الخلايا الجريبية للغدة الدرقية والدور الوقائي المحتمل لكبريتات الزنك في ذكور الفئران البيضاء البالغة

أسماء البنا و فاطمة بدر الدين^١ و رحاب أحمد عبد المنعم^٢ و فاطمة دويدار^٣

مقدمة: الأروكلور ١٢٥٤ عبارة عن خليط تجارى من مواد ثنائي الفينيل متعدد الكلور يستخدم في المحولات ومواد الطلاء والمبيدات والتلفاز ومكيفات الهواء. وتأثير الأروكلور على الغدد الصماء وخاصة الغدة الدرقية محل اهتمام البحث **الهدف من هذه الدراسة:** هو فحص التأثير السمي المحتمل للأروكلور ١٢٥٤ على الخلايا الجريبية للغدة الدرقية في ذكور الفئران البيضاء البالغة وأيضا تقييم الدور الوقائي المحتمل لكبريتات الزنك.

مواد وطرق البحث: تم إجراء هذا البحث على ٤٥ من ذكور الفئران البيضاء البالغة ، تم تقسيمهم عشوائيا إلى ثلاث مجموعات متساوية (١٥ فأر لكل مجموعة): المجموعة I (المجموعة الضابطة): قسمت إلى ثلاثة مجموعات داخلية Ia, Ib, Ic والتي أعطيت ماء مقطرا و زيت الذرة أو كبريتات الزنك علي التوالي؛ المجموعة II: تعاطت أروكلور ١٢٥٤ بجرعة ٤ مجم/كجم مذابة في زيت الذرة؛ المجموعة III: تعاطت كبريتات الزنك بجرعة ٢٠٠ مجم/كجم مذابة في ماء مقطر في نفس الوقت مع أروكلور ١٢٥٤ بنفس الجرعة السابقة. وقد استمرت مدة البحث ثمانية وعشرون يوما. في

TSH T4 T3

نتائج البحث: نتائج التحاليل الكيميائية انخفاضا ملحوظا في مستوى الجلوتاثيون إجمالي السعة المضادة للأكسدة T3, T4 في المجموعة الثانية مقارنة بالمجموعة الضابطة و المجموعة الثالثة. TSH في المجموعة الثانية عن المجموعتين A

للمجموعة التي تعاطت الأروكلور ظهور جريبات الغدة الدرقية بحجم اصغر من المجموعة الضابطة و مبطنة في بعضها بأكثر من طبقة من الخلايا الجريبية. و جريبات اخري مبطنة بخلايا جريبية مستطيلة و بها بفجوات في جيلة الخلايا. ظهرت المادة الغروية بشكل مجوف في منطقة التقائها بالخلايا. : أظهرت تحسنا ملحوظا في الشكل الهستولوجي. : ظهرت بعض الخلايا الجريبية بشكل مستطيل كما أظهرت الشبكة الاندوبلازمية الخشنة اتساعا شديدا وزيادة في جسيمات الحالة وظهرت بعض النواة منكمشة وتوزيع الكروماتين بها غير طبيعي. : أظهرت تحسنا ملحوظا في الشكل الهستولوجي.

الاستنتاجات: إلى مركبات ثنائي الفينيل متعدد الكلور سببت الأكسدة وكذلك قصور الغدة الدرقية في كبريتات الزنك دور وقائي إلى زيادة تعويضية في نشاط الغدة في صورة زيادة طب

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٣ قسم الكيمياء الحيوية - كلية الطب - جامعة الإسكندرية